THE HAEMORHEOLOGY OF TRANSIENT ISCHAEMIC ATTACKS

by

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SUMMARY

Stroke is a frequent cause of death and disability in Western society for which therapy has little to offer. The limitations of therapy for completed stroke has placed great emphasis on stroke prevention and this is manifested mainly by the elimination and control of previously identified risk factors, of which hypertension is the prime example. The occurrence of a transient ischaemic attack (TIA) is an important risk factor because, although only a minority of strokes are preceded by such an attack, the incidence of completed stroke following a TIA is high, particularly if the attack occurs in the carotid territory. In a majority of patients who experience a TIA the cause of the attack is identified; thromboembolic disease is the commonest aetiology and extracranial arterial disease is the source of emboli in most of these patients. In a minority of patients the cause of their symptoms remains undiscovered, often in spite of extensive investigation. The studies in this thesis set out to determine if TIAs of unknown aetiology shared the poor prognosis of thromboembolic attacks, and if so whether haemorheological abnormalities could explain these attacks.

A retrospective follow-up study of patients with carotid distribution TIAs, in whom no embolic source had been identified by routine screening procedures, indicated that the stroke risk in this group is almost as high as that observed in studies of the natural history of TIA. In the light of this finding it was felt important to attempt to discover the cause of the symptoms in this group of patients with a view to developing a rational management policy. TIAs are the result of focal cerebral perfusion failure which may

theoretically be due to inadequate perfusion pressure, diminished vessel radius or increased blood viscosity. Each of the first two mechanisms has been previously implicated in the pathogenesis of TIA. Consequently it seemed reasonable to investigate the possible rôle of increased blood viscosity in unexplained TIAs.

A prospective study of whole blood viscosity, and its determinants, in carotid territory TIA revealed that there are no differences in whole blood viscosity between patients with unexplained attacks and those with presumed thromboembolic disease, identified by Doppler carotid scanning. Plasma fibrinogen levels were significantly higher in patients with carotid disease compared to those without detectable carotid disease. A study to compare haemorheological parameters in TIA patients with a normal age and sex-matched control group, demonstrated that although whole blood viscosity was the same in both groups plasma viscosity and plasma fibrinogen were both significantly higher in the TIA group. Further studies revealed that these changes in plasma viscosity and fibrinogen are similar in magnitude to those found in peripheral arterial disease patients, who have well documented blood viscosity abnormalities.

Elevations in plasma fibrinogen levels may occur for many reasons. With regard to cerebrovascular disease the most relevant chronic influences on the plasma fibrinogen level are the presence of hypertension and cigarette smoking, both of which may cause it to rise. The possibility that the elevated fibrinogen levels in TIA patients, particularly those with carotid disease, were due to an increased prevalence of smoking and hypertension in these groups was considered. Sub-group analysis revealed that the influence of

clinically evident arterial disease, manifested either by symptoms or positive Doppler carotid screening, outweighed the effect of smoking and hypertension.

In conclusion, TIA patients who do not have a detectable embolic source are at a risk of stroke and cardiovascular morbidity comparable to other patients with TIA, and therefore merit continued review and/or repeated or more extensive investigation. Viscosity abnormalities do not account for symptoms in these patients. However, fibrinogen levels are elevated in all TIA patients, most notably those with carotid disease. In view of the association between raised plasma fibrinogen and arterial disease it is possible that patients with unexplained symptoms have undetected arterial disease which acts as a source of emboli.

It is accepted that fibrinogen is a risk factor for ischaemic heart disease and stroke, and these studies now show that it is associated with TIA and the degree of detectable carotid disease. The relationship between raised fibrinogen and atheroma may be causative, consequential or merely coincidental. If increases in fibrinogen follow the development of arterial lesions or are produced by common stimuli then fibrinogen levels act only as markers of disease. If however, raised fibrinogen levels promote atheroma production or predispose to thrombosis in relation to pre-existing atheroma then defibrinogenation therapy may be beneficial in individuals at risk from arterial disease. Studies on the efficacy of such therapy must await the development of more suitable drugs than are available at present.

Chapter 1

INTRODUCTION: STROKE AND TRANSIENT ISCHAEMIC ATTACK

Stroke is an important cause of morbidity and mortality in developed countries; in most of the western world it is exceeded as a cause of death only by heart disease and malignant disease (1,2). Both socially and economically the burden of stroke morbidity is high and although most of those afflicted are elderly, up to one third may be of working age (3). The Framingham Study demonstrated that almost three-quarters of stroke survivors suffered long term disability and 16% were permanently hospitalized (4). With this effect on the community it is not surprising that stroke therapy and prevention are the focus of much attention and research.

Brain infarction is irreversible and therapy for completed stroke is limited to minimising the area of infarction, rehabilitation of the patient and prevention of stroke recurrence. The inadequacy of therapy for completed stroke, and the potentially catastrophic effects of this event, have meant that great effort has been directed towards identifying and eliminating risk factors for this disease in the hope of reducing its incidence. Stroke is a heterogeneous condition comprising thromboembolic brain infarction, intracerebral haemorrhage and subarachnoid haemorrhage, accounting for 69-80%, 10-12% and 7-8% respectively, of all strokes (3,5). Risk factors are different for each of these conditions but on the basis of numbers alone it is obvious that those related to thromboembolic disease are the most relevant. The most important of these is hypertension and it is also a risk factor for intracerebral haemorrhage. The significance of

hypertension as a risk factor is related not only to its strong correlation with cerebral infarction but also to its high prevalence. Control of hypertension is an essential factor in stroke prevention and successful pursuit of this goal has been postulated as one reason for the diminishing stroke rate (6).

Figures for many European countries, as well as the United States of America, demonstrate that the incidence of stroke is declining, and in some countries has been declining for many years (2,7,8). The identification and control of hypertension in the community may have contributed to the apparent acceleration in this decline in recent years (6,9) but cannot account for changes which have been taking place for decades; figures from the USA for example indicate that the incidence of stroke has been decreasing since 1900 (2,7,8). Similarly, modifications in lifestyle such as stopping smoking, increasing exercise and decreasing dietary fat intake, designed to lessen the incidence of arterial disease generally, can also be responsible only for recent changes.

In spite of the effects which hypertension control and other changes may have had on stroke incidence it remains a major problem. One overwhelming difficulty is that stroke occurs in the majority of cases without warning and under these circumstances only screening the general population to detect and eliminate risk factors will be of benefit. In a minority of individuals however, completed stroke is preceded by a transient ischaemic attack (TIA) and the occurrence of such an event presents the attending physician with an opportunity to prevent its progression to cerebral infarction. There is disagreement about the proportion of completed strokes which are preceded by one or more TIAs; estimates range from 10% to 50% (5,10,11). Likewise, studies on the natural history of TIA show a wide variation in the

incidence of subsequent stroke (table 1.1). There are several possible reasons for these wide variations. All studies of the natural history of TIA were carried out several years ago and will not be repeated, since no TIA patients are now left untreated. It is likely that many of the groups studied were heterogeneous for two reasons: firstly, a uniform, widely accepted definition of TIA was not agreed until relatively recently; secondly, the absence of adequate assessment of intracranial disease by computed tomography resulted in the inclusion of patients with non-vascular cerebral diseases.

However it is now generally accepted that transient cerebral ischaemia carries a greatly increased risk of subsequent stroke (19,20). It has been estimated that of the patients who develop stroke in the first five years after an initial TIA 20-24% will do so in the first month and 42-50% in the first year (17,21). After this period the clinical risk is 5-7% per annum (21,22) which may be up to four or five times the risk in a control population (19,20). It is clear that this group of patients may benefit from investigation and treatment of whatever underlying disorder predisposes them to stroke.

In the majority of cases this underlying disorder is arterial disease (See chapter 2.2). Like arterial disease which presents in other sites it is not a localised disorder, a fact which is borne out by the high mortality from coronary disease suffered by patients who present with TIAs. Indeed, although the incidence of stroke is high in these patients, the commonest cause of death is myocardial infarction (17,18,23). The concept that TIA may be an early manifestation of otherwise subclinical arterial disease will be discussed at some length in this thesis.

Author	Year	No. strokes/patients(%)	Follow up
ACHESON (12)	1964	42/82 (51.2%)	39 months (mean)
MARSHALL (13)	1964	4/180 (2%)	3 months - 7 years
PEARCE (14)	1965	2/20 (10%)	10.6 months (mean)
BAKER (15)	1968	17/79 (22.5%)	41 months (mean)
ZEIGLER (16)	1973	22/135(16.3%)	36 months (mean)
CARTLIDGE (17)*	1977	85/199(42.7%)	5½ - 20 years
TOOLE (18)	1978	11/56 (19.6%)	3 - 14 years

*Some patients treated

TABLE 1.1

NATURAL HISTORY OF TIA : STROKE RISK

Treatment of patients with TIA is controversial and randomised trials of surgical and medical management are in progress. In addition, the assessment of these patients by non-invasive means is the focus of much research. The initial aim of this thesis was to contribute to the management of this problem by studying a small group of TIA patients in whom no obvious cause of their symptoms could be identified using current techniques of investigation; and in whom, consequently, appropriate therapy could not be instituted. It was thought that abnormal blood viscosity might be important in the genesis of their symptoms. Exploration of this hypothesis led to an extensive evaluation of haemorheology and its relation to risk factors in patients with TIA. Before the possible relevance of haemorheology in TIA may be discussed it is necessary to define the pathophysiology and diagnosis of TIA in greater detail than has been done so far, and these will be discussed in the next chapter.

Chapter 2.

PATHOPHYSIOLOGY AND DIAGNOSIS OF TIA.

2.1 DEFINITION

Variable definition of TIA has been one reason for the different conclusions reached in various studies of the pathogenesis and natural history of this syndrome (12-18). In recognition of this, an ad hoc Committee for the Classification of Cerebrovascular Disease, set up by the Advisory Council for the National Institute of Neurological and Communicative Disorders and Stroke, published definitions covering TIA and allied disorders in 1975 (24). These definitions are now widely accepted. TIAs are defined as "episodes of temporary and focal cerebral dysfunction of vascular origin, rapid in onset, which are variable in duration, commonly lasting from two to 15 minutes but occasionally lasting as long as a day". All attacks resolve with no residual deficit. Episodes which last longer than 24 hours but resolve completely in one week are prolonged reversible ischaemic neurological deficits (PRIND) and differ from TIAs only in their duration. Any deficit which lasts for more than a week is defined as a stroke. It must be appreciated that these time limits are arbitrary and do not imply any differences in aetiology or necessarily delineate groups for subsequent treatment. Consequently, as will be discussed later (chapter 2.2), there is considerable heterogeneity within groups with regard to pathophysiology.

The definition of TIA uses symptomatology as its sole criterion and the diagnosis, therefore, must depend on the history. The duration of most attacks is less than 15 minutes and most signs will have resolved completely by the time a patient obtains medical attention. It is important to elicit precise details of an attack to allow differentiation from other syndromes which may mimic TIA. Migraine, for example, may produce hemiplegia but a careful history should identify patients whose attacks are accompanied by headache or are characterised by gradual onset of symptoms, thus excluding them from consideration as TIAs. It is more difficult to exclude other causes of focal symptoms by history alone and even clinical examination may contribute little.

The vascular supply of the brain is derived almost entirely from the circle of Willis at the base of the skull and this is supplied in turn by the vertebral and carotid arteries. TIAs are described either as carotid or vertebrobasilar in distribution depending on which part of the circulation appears to be predominantly affected. This anatomical differentiation is associated with clinical and pathophysiological differences between attacks in the two areas.

Diagnosis of vertebrobasilar ischaemia (VBI) is more difficult because the symptoms commonly encountered, such as vertigo and unsteadiness, occur in a wide range of other conditions and are often not related to cerebrovascular disease. These symptoms may also be vague and, in recognition of this, VBI is not diagnosed on the basis of vertigo, diplopia, dysarthria, or dysphagia alone, but only if these symptoms occur in concert with other diagnostic events such as homonymous hemianopia or motor or sensory deficit. Attacks in the carotid territory are often more definite events and are less likely to be confused with anything except significant cerebral disease.

Perhaps the most important difference between carotid TIA and VBI is that the latter is said to carry a lesser risk of subsequent stroke (13,15,16,25). This may be allied to differences in pathophysiology between attacks in the two systems and is something which will be discussed further (chapter 2.3). Because of this perceived difference in stroke risk, surgical treatment of VBI in the form of vertebral artery surgery is rare, in contrast to carotid surgery which is widely practised.

The investigation of symptomatic patients to exclude non-vascular causes of TIA and to identify the likely aetiology of an attack will be discussed later. An understanding of the pathophysiology of TIA is an important prerequisite for the appreciation of the methods used to investigate these patients and a discussion of this follows.

2.2 PATHOPHYSIOLOGY

Several pathophysiological mechanisms have been advanced to explain TIAs with varying degrees of acceptance. These include arterial spasm, haemodynamic abnormalities, embolism and haematological abnormalities and each of these will now be considered in turn.

A. Spasm.

What was perhaps the first attempt to explain TIAs was published as long ago as 1891 when vascular spasm was invoked as an explanation for transient cerebral ischaemic symptoms. Peabody reported a case of recurring right hemiparesis in a 56 year old man in whom, at post mortem, he was unable to detect any signs of cerebral infarction (26).

He attributed these symptoms to spasm of the middle cerebral artery which was of sufficient duration to cause ischaemia but not sufficient to lead to infarction. Evidence to support this suggestion is lacking.

Vasospasm is a feature of migraine and may be seen on angiograms carried out during an attack of hemiplegia attributable to this disease (27). Apparent areas of spasm have been observed in vessels in models of hypertensive encephalopathy, but this is most likely to be a local reactive phenomenon in response to leakage of plasma from damaged vessels. Harrison mentions several cases of TIA in whom the blood pressure was found to be grossly elevated during a TIA and suggests that reactive vasoconstriction as a response to increased perfusion pressure may be relevant (28). No evidence exists to suggest that this reflex spasm leads to focal ischaemia and this pathophysiological mechanism remains unproven.

B. Haemodynamics.

Decreased cerebral perfusion almost invariably leads to global rather than focal symptoms. This was demonstrated by Kendell and Marshall in 1963 when they studied 37 patients with TIA (29). Attempts to reproduce their focal symptoms by manipulation on a tilt table following the administration of ganglion blocking drugs failed in all but one case. Examination of patients during TIAs has also shown that few are hypotensive at the time of the attack (30).

If haemodynamic events were important in the pathogenesis of TIA then some association between manoeuvres likely to produce hypotension and the onset of symptoms should be apparent. While there have been isolated cases reported where links between onset of TIA and precipitating factors have been apparent, these are most unusual (31).

Cardiac arrhythmias may produce systemic hypotension and might be expected to produce symptoms due to cerebral hypoperfusion. Again these symptoms tend to be global rather than focal. In 290 patients requiring pacemaker insertion for cardiac arrhythmias Reed et al found only four who described focal cerebral symptoms, while global cerebral symptoms were common, occurring in 235 patients (32). In another report of cardiac arrhythmia associated with cerebral ischaemia, none of the patients studied experienced focal symptoms (33). A controlled study of possible cardiac causes of TIA found that the incidence of arrhythmias in the control population was as high as in the group of patients with TIA (34).

In unusual circumstances reduced cerebral perfusion pressure may lead to focal signs and this was the case in the patient described by Eastcott in the first European report of a successful carotid reconstruction (35). This patient experienced recurrent hemiplegic attacks and amaurosis fugax coincident with episodes of cardiac arrhythmia. Angiography demonstrated a right carotid stenosis ipsilateral to the affected hemisphere. Surgical correction of the stenosis by excision and end to end anastomosis caused resolution of the cerebral symptoms, although the arrhythmias persisted postoperatively. Ruff observed several patients who became hypotensive in the course of arteriography and only those with a haemodynamically significant carotid stenosis developed focal neurological deficits (36).

In summary, therefore, it appears that hypotension, however caused, will only provoke focal cerebral symptoms in unusual circumstances eg, when a tight arterial stenosis renders a region selectively vulnerable to a drop in perfusion pressure. This mechanism probably accounts for only a small proportion of all TIAs.

C. Embolism

Denny-Brown in 1960 related TIA to stenosis or occlusion of at least one major cerebral artery and proposed that it could be precipitated by a decrease in blood pressure, anaemia or increased blood viscosity. He also postulated that recovery from the attacks depended upon collateral circulation and that cerebral emboli may be important in their pathogenesis (37).

Shortly before this in 1959 Fisher had published an account of observations of the retinal vessels in a patient during the course of an attack of amaurosis fugax (38). White material was observed in the vessels obstructing bifurcations then gradually fragmenting and moving more distally. Obstruction of the retinal vessels corresponded to the pattern of visual field loss and, as the embolus fragmented, vision gradually returned. These observations were confirmed two years later by Ross Russell (39). This was the first direct evidence that symptoms of transient vascular obstruction in the retina were due to emboli and not to vascular spasm. It was considered that such events were unlikely to be confined to the visible vessels of the retina and similar embolic occlusion of the cerebral vessels could be inferred to explain other TIAs. At the time of their initial observations Fisher and Ross Russell were unaware of the nature of the substance which constituted the embolic material. Later, McBrien and his co-workers demonstrated from pathological studies that these emboli in the retinal arteries were made up of platelet aggregates (40).

Most of the evidence in favour of embolism as a cause of TIA is indirect. For example, the occurrence of atheroma at the carotid bifurcation is much more frequent in TIA patients than in controls. Harrison and Marshall compared angiograms from patients with completed

stroke, TIA, and cerebral tumour, and noted that atheroma sufficient to cause stenosis at the origin of the internal carotid was present in 40% of patients with hemispheric TIA, but in only 3% of patients with cerebral tumour (41). The degree of stenosis at the carotid bifurcation is rarely sufficient to cause reduction in flow. It has been demonstrated in a carotid model that only when the cross sectional area of a stenosis is less than two square millimetres, is reduction in flow inevitable, and a stenosis which leaves a luminal cross sectional area greater than $5mm^2$, never produces decreased flow, provided pressure is maintained (42). In a medium-sized internal carotid of 10mm diameter, a cross sectional area of $5mm^2$ is achieved with a stenosis greater than 80% and a $2mm^2$ area with a stenosis greater than 90%. When atheroma produces stenosis less severe than this, the association between it and TIA presumably depends on its ability to produce emboli.

A number of studies have shown that carotid endarterectomy performed soon after a TIA is more likely to demonstrate thrombus in association with atheroma than if it is performed after a greater time interval. In an initial study of 12 patients , Gunning reported that thrombus was present if endarterectomy was carried out within six or seven weeks after a TIA, but not if the interval was longer (43). Harrison and Marshall's series of 52 patients demonstrated thrombus in 66% of cases operated on within four weeks of a TIA but in only 21% of those treated later then this (44). Thrombus formation in association with atheroma is an episodic event which appears to be temporally related to the occurrence of TIA.

The chance of finding thrombus in relation to a carotid atheromatous plaque also varies with the degree of stenosis produced, ie, with the size of the plaque (44). In tightly stenosed lesions, 60%

will have thrombus attached, while stenoses of between 25% and 70% will be associated with thrombus in only 50% of cases. Lesser degrees of stenosis are associated with thrombus in only 10% of cases. Large atheromatous plaques are more complex structures than smaller lesions and often contain areas of haemorrhage and ulceration, which accounts for this association with thrombus formation (45). The more benign prognosis of minor stenosis in terms of stroke risk also fits well with these observations (46). Small plaques carry a poorer prognosis if they are ulcerated (47) and carotid endarterectomy performed in patients with ulcerated, non stenotic plaques, produces relief of symptoms in the majority of cases (48).

Further evidence relating microembolic events to TIAs is available from early angiographic study of such patients. In 25% of patients who have angiography carried out within 48 hours of a TIA or minor stroke, occlusion of small intracerebral arteries may be demonstrated. These obstructions are not demonstrable on subsequent arteriograms in the same patients (49,50).

More support for the embolic theory of TIA pathogenesis is provided by the demonstration of an increased incidence of TIA in patients with valvular heart disease. Rheumatic heart disease is not uncommonly associated with embolism to the brain, retina, and other organs and these emboli may give rise to clinically evident episodes of ischaemia. In a series of 325 patients with rheumatic heart disease, Hutchinson identified 44 (14%) who had experienced classic cerebral embolic episodes (51). Half of these patients had hemispheric symptoms and one quarter experienced amaurosis fugax; the remainder suffered vertebrobasilar attacks. Amaurosis fugax is commonly associated with rheumatic heart disease, occurring in up to one-third of patients, and is unrelated to the presence of arrhythmias (52). The

absence of association with arrhythmia has led to the conclusion that emboli originate from the abnormal valve leaflets and not from the chambers of the heart. Emboli arising from the atria or ventricles secondary to fibrillation or myocardial infarction, are often implicated in the pathogenesis of stroke. These emboli are larger and more stable than those originating from the valve leaflets or from ulcerated atheromatous plaques. A possible explanation of these observations is that small friable emboli cause TIAs and larger stable emboli cause completed strokes.

The link between rheumatic heart disease and TIA has long been established but in recent years attention has been focused on less well recognised disease of the heart valves as a source of emboli. Myxomatous degeneration of the mitral valve (prolapsing mitral valve, floppy mitral valve) was recognised as a distinct entity in the late 1950s and early 1960s. In 1975 it was established that it was a common abnormality with an incidence of 5-10% in the general population (53,54). Barnett first drew attention to this condition as a cause of TIA in 1974 with a report on four patients, and followed this with other, larger series of cases (55-57). Subsequently he found that mitral valve prolapse occurred in 40% of patients under 45 years of age with TIA or stroke, but in only 6.8% of an age and sex matched control group (58); he concluded that it was a significant source of emboli in young patients in whom the incidence of atheroma was low.

Mitral annulus calcification is another long recognised condition which has only relatively recently been shown to be relevant in the pathogenesis of TIA. De Bono found that eight patients in a series of 151 with cerebral or retinal ischaemia had this valvular defect, but found no case among 188 matched controls (59). In four of these cases, emboli were visualised in the retina and these had appearances

consistent with calcified debris. These clinical studies of valvular disease have been supported in their conclusions by evidence from pathological studies which have shown that thrombus does form on abnormal valve cusps and may subsequently embolise (60,61).

The evidence in favour of embolism as a cause of TIA in the majority of cases is strong and potential sources of emboli may be found in 75% of patients with transient retinal or cerebral ischaemia (34). There are, nevertheless, a number of situations which are not entirely satisfactorily explained by the embolism theory. Many patients experience multiple TIAs which may be stereotyped, and some authors object to the embolic theory as an explanation of these attacks on the grounds that it is difficult to imagine recurrent emboli consistently finding their way into the same vessel. In answer to this, Harrison points out that careful history taking will demonstrate that attacks, which at first appear identical, have subtle differences which may indicate emboli occluding adjacent but separate cerebral arterial branches (62). Others point to the work by Honour in which repeated emboli from cerebral vessels in rabbits could be shown to enter the same distal vessel (63).

One frequently observed clinical event which casts doubt on the theory that embolism can explain all TIAs, is the occurrence of symptoms in the distribution of an occluded vessel. In many patients with recurring TIAs in the carotid distribution the attacks cease, without stroke, when the vessel occludes. Some patients, however, will continue to experience transient ischaemic symptoms in the distribution of such an occluded vessel. If these result from emboli, then these emboli must find their way to the appropriate circulation via extra-cranial to intra-cranial collaterals, or across the circle of Willis. A study of these patients has revealed that a majority have

significant disease in the vessels supplying the collateral channels which fill the circulation distal to the occlusion, ie, in the proximal common carotid or the external carotid. It has also been postulated that emboli could arise from the proximal "stump" of the occluded internal carotid and similarly make their way via collaterals (64). Yet another explanation is that the distal "tail" of thrombus in an occluded internal carotid may release emboli into the circle of Willis. These explanations are not entirely satisfactory and the cause of symptoms in these patients remains unproved.

D. Haematological abnormalities

Neurological manifestations of haematological disease have been recognised since early this century, and in 1960 Millikan et al described carotid and vertebrobasilar insufficiency as a consequence of polycythaemia in 22 patients seen at the Mayo clinic (65). The symptoms described in these patients were clearly specific neurological events and distinct from such vague symptoms as dizziness, blurred vision, and headache, often reported by patients with polycythaemia. Polycythaemic patients have been extensively investigated by Thomas and his co-workers who have shown that cerebral blood flow (CBF) varies inversely with haematocrit (66,67). In patients with polycythaemia, low CBF may promote stasis in small vessels and give rise to transient obstruction. A high haematocrit also promotes thrombus formation due to interactions between red cells and platelets which are enhanced in polycythaemia by the high platelet count.

Anaemia as a cause of TIA has also been reported in the literature but has received less attention (68). If diminished oxygen supply to the brain is postulated as a mechanism, then some

explanation of the focal nature of the symptoms is required. In the series of five patients reported by Seikert (68), at least two had significant atheroma and it seems likely that a region of reduced perfusion is essential if anaemia is to produce focal rather than global symptoms. Anaemia is thus akin to hypotension as a cause of TIA; a predisposing pattern of atherosclerosis is required to produce focal symptoms.

Other unusual haematological abnormalities have been reported as rare causes of TIA. Severe leucocytosis in leukaemic patients has been identified as a cause of vertebrobasilar ischaemia, presumably as a result of the obstruction of small vessels by large numbers of white cells (69).

The possibility that less readily detectable haematological abnormalities, ie, those producing viscosity changes, may have a rôle in the genesis of TIAs is a major topic of discussion in this thesis and will be considered in detail later.

Summary

It is now generally accepted that the commmonest and most important cause of TIA is embolism from the proximal vasculature. The carotid bifurcation is the commonest site of atheroma in the cerebral circulation (70) and is implicated as the source of emboli in most patients (34). Some patients undoubtedly embolise from other vessels and from diseased heart valves and this possibility must be borne in mind during the investigation of transient ischaemic symptoms. In a small minority of patients haematological abnormalities may account for their symptoms and the diagnosis is established by a simple blood count.

2.3 DIAGNOSIS

Clinical History

The importance of the history in the diagnosis of transient ischaemic attacks cannot be overemphasised. The short duration of most attacks and the absence of clinical signs following resolution means that the physician is unlikely to witness anything of relevance and must rely on the testimony of the patient and any other witnesses to the event. Perhaps surprisingly this does not mean that it is a difficult diagnosis to make but care must be taken to avoid confusion with a number of other neurological disturbances.

Attacks are normally divided into those affecting the posterior circulation (vertebrobasilar), and those affecting the carotid distribution (hemispheric). The pattern of symptoms produced in each area is usually characteristic. The importance of this separation of attacks into vertebrobasilar and carotid is threefold. Firstly, vertebrobasilar ischaemia (VBI) is believed to carry a less serious prognosis in terms of the likelihood of subsequent stroke (13,15,16). Secondly, the aetiology of attacks in different areas may vary significantly. For example, haemodynamic disturbance due to kinking of vessels in the neck is thought to be important in many instances of VBI but it is a rare cause of carotid TIA (71-73). Finally, in view of the first two reasons, and the relatively inaccessible nature of the vertebral arteries, surgical intervention is reserved largely for the carotid vessels.

Carotid TIAs are characterised by rapid onset of symptoms, usually maximal in less than two minutes, which commonly affect the motor or sensory distribution on one side. Symptoms may be purely

motor or sensory or a combination of both. Absent or decreased motor function may be manifest as clumsiness, weakness, or incoordination. Sensory impairment alone may be less striking since function is normal; numbness or paraesthesia affecting the arm and/or ipsilateral leg may be the sole abnormality. Dysphasia may be a feature of carotid attacks involving the left hemisphere but this symptom occurring alone is not diagnostic of a TIA. Homonymous hemianopia may occur but is an unusual symptom in carotid distribution TIA. Transient unilateral blindness (amaurosis fugax) is an important and common presentation of carotid attacks. The blindness may be complete or partial affecting only the upper or lower visual fields, and the patient frequently describes the onset as being like a shutter or blind being lowered or raised. Resolution occurs in five to ten minutes and the entire episode is painless (24).

The clinical features of vertebrobasilar attacks are often less precise than carotid TIAs and VBI is consequently more readily confused with other disorders, but the history will still be adequate for diagnostic purposes in nearly all cases. Like carotid attacks, symptoms are rapid in onset and typically last less than 30 minutes. Motor and sensory symptoms need not be limited to one side and unilateral symptoms may affect different sides in different attacks. Drop attacks may occur and visual symptoms include complete or partial blindness and diplopia. Vertigo, dysarthria and dysphagia may accompany attacks but none of these symptoms occurring alone should be considered diagnostic of ischaemia (24). The duration of TIAs in both circulations is similar; symptoms resolve in the majority of cases within half an hour but may last up to 24 hours.

Examination

Clinical examination of patients with a history of transient cerebral ischaemia is concerned mainly with detection of causes of the ischaemia and exclusion of other disorders which may mimic TIA; examination has almost no part to play in the diagnosis of the ischaemic event itself, except in the unlikely event of the attack being witnessed. Since the pathogenesis of TIA is intimately related to cardiovascular disease, examination of the cardiovascular system as a whole may yield information on the aetiology of TIA. Examination of peripheral pulses may show deficits indicative of widespread vascular disease but specific examination of the carotids is of little benefit. Palpation of pulses in the neck rarely yields useful information; severe stenosis or occlusion of the internal carotid artery may exist in the presence of a palpably normal carotid pulse. Auscultation of the cervical region for bruits is notoriously inaccurate in the detection of carotid disease (74). Audible bruits can originate not only from the internal carotid, where their presence may indicate clinically relevant disease, but also from the external carotid and the heart. Conversely, severe internal carotid stenosis may be present without a detectable bruit and an occluded vessel never produces a bruit.

The heart is frequently a source of pathology which may be relevant in TIA, and particular attention should be paid to the rate, rhythm, and character of the pulse, the detection of abnormal precordial signs and sounds, and the blood pressure in both arms. The presence of arrhythmias, or murmurs indicative of valvular disease can signify possible embolic sources. The recognition (and treatment) of hypertension is also important in any patient with symptoms of

cerebrovascular disease in view of the well defined risk association. Examination of the optic fundus may reveal bright intravascular plaques, first described by Hollenhorst (75), which are believed to be cholesterol crystals which have arisen from ulcerated atheromatous plaques. Rarely, during attacks of amaurosis fugax, pale platelet emboli may be seen traversing the retinal vessels, and calcified emboli from heart valves have been identified in the retinal circulation (59).

2.4 INVESTIGATION

Laboratory investigation of TIA patients should include a full blood count, serum biochemistry, blood glucose and plasma lipids. An electrocardiograph (ECG) will assist in detection of relevant cardiac disease and indicate which patients should have more detailed cardiac assessment using echocardiography or 24 hour ambulatory ECG monitoring. It is now accepted that these latter tests have little place in the evaluation of TIA patients as a group but rather have an important role to fulfil in the further investigation of individuals selected on the basis of abnormal physical examination or ECG abnormalities (76-79). In view of the higher proportion of younger patients shown to have cardiac abnormalities some authors have suggested that these investigations should also be carried out routinely in patients under the age of 45 who present with TIA or stroke (80,81).

Atheroma of the extracranial carotid artery is found in 31-78% of patients with carotid distribution TIAs (34,50,82-85); evaluation of the carotid vessels is therefore an important part of the assessment

of these patients. Traditional intra-arterial angiography is the most direct and still the most accurate method of displaying the carotid circulation but it is invasive with a definite morbidity. In an attempt to diminish the demand for and therefore the morbidity from arteriography, many non-invasive tests which detect disordered carotid circulation have been devised in the past ten years. Non-invasive assessment of the carotid vasculature may be direct or indirect. Direct tests rely on information gained directly from the carotid vessels at, or close to, the common carotid bifurcation; indirect tests assess changes in the more distal carotid circulation. A brief review of the most relevant tests with a discussion of their underlying principles and current usefulness follows.

Indirect tests

Ophthalmodynamometry

This technique was developed early this century and involves increasing intraocular pressure to the point where blanching of the ophthalmic artery, observed through an ophthalmoscope, indicates that intraocular and ophthalmic arterial pressures are equal. It was developed initially to measure pressure in the retinal artery which is a direct branch of the internal carotid; changes in its pressure often mirror changes in carotid artery pressure. Stenosis or occlusion of the internal carotid on one side may produce a lowering of the ipsilateral retinal artery pressure. A difference between sides of 15% or more strongly suggests internal carotid disease (86). There are limitations to the usefulness of this examination as a screening test for carotid disease. Firstly, it will only detect high grade stenosis or occlusion; lesser degrees of stenosis or ulcerated plaques which may be of diagnostic relevance will be overlooked. Secondly, even in

patients with occlusion or high grade stenosis, collateral circulation may develop sufficiently to equalise pressure in the retinal arteries and no abnormality will be apparent. Similarly bilateral carotid disease may produce equal lowering of pressure on both sides and yield an apparently normal result. For these reasons this investigation has been largely superseded in the detection of carotid artery disease.

Air oculoplethysmography (OPG)

Air OPG is a more objective method of measuring ophthalmic artery pressure. Small suction cups are applied to the sclera and an increasing vacuum applied. This causes a rise in intraocular pressure which is proportional to the vacuum. The suction cups are also connected to a pulse detector which records ophthalmic artery pulsation. Pulsation ceases when intraocular pressure exceeds ophthalmic artery pressure. Difference between the sides suggests carotid disease. The drawbacks of this investigation are similar to those of ophthalmodynamometry. In an effort to overcome these, various manoeuvres, including carotid compression and calculation of the ratio of ophthalmic artery to brachial artery pressure have been recommended to improve the diagnostic accuracy of this procedure (87). Despite this the diagnostic usefulness of this test remains limited to the detection of occlusion or near occlusion and even so it may not be able to distinguish between these two states.

Fluid oculoplethysmography

Pulsation of the eyeball during systole provides the basis for the more sophisticated technique of fluid oculoplethysmography. Systolic expansion is detected by fluid filled scleral cups; the waveform, timing and amplitude of the pulse may be studied. Unilateral carotid disease is detected by comparing the right and left eyes and

bilateral stenoses by comparing the ocular pulse with the ear pulse via photoplethysmographic sensors on the ear lobe. Using this technique alone stenosis of greater than 40% in the internal carotid may be detected with an accuracy of 87% (88). This technique shares a disadvantage common to all indirect tests which is that even when abnormalities are detected, no information on their location is available. This may be improved by combining this investigation with phonoangiography, discussed below.

Supraorbital Doppler

The supraorbital artery is a branch of the ophthalmic artery which takes part in the superficial temporal anastomosis with the superficial temporal artery. Normal flow in the supraorbital artery is directed out of the orbit, but in cases of internal carotid stenosis this flow may be reversed. Under these circumstances compression of the ipsilateral superficial temporal artery, instead of resulting in increased flow as would normally be the case, leads to diminished, abolished or reversed flow in the supraorbital artery. These changes in flow may be detected by a directional Doppler flow probe placed over the supraorbital artery. This technique is limited to the detection of stenoses of 75% or more and even in these circumstances gives rise to many false positive and false negative results (89).

Supraorbital photoplethysmography is an allied technique which utilises photoplethysmographs rather than Doppler probes to measure pulsation in the relevant vessels. It appears to be no better than the Doppler method in terms of diagnostic accuracy (90).

Direct tests

Carotid phonoangiography

The limited value of auscultation of the neck in the detection of carotid bruits has already been mentioned. Phonoangiography is an extension of simple auscultation which attempts to overcome these limitations by using a microphone to record bruits in the neck thus permitting analysis of their quality, timing and duration (91). A bruit which lasts for 75% of systole is associated with stenosis of 50% or more and one which continues into diastole indicates a stenosis of 80-90%. The value of this investigation is limited by interference from bruits from the external carotid and by the fact that a tight stenosis (greater than 90%) may produce no bruit. It is also impossible to distinguish between occlusion and a normal vessel. These limitations are overcome by combining this investigation with fluid oculoplethysmography.

Doppler Techniques

A brief review of the use of Doppler detectors in the investigation of carotid disease is given here. A more extensive discussion of the principles, execution and usefulness of Doppler imaging and spectrum analysis is given in chapter five.

1. Imaging.

Pulsed ultrasound may be used to produce an image of blood vessels on an oscilloscope screen. The apparatus required to do this consists of a Doppler probe which both generates and receives ultrasound signals, and a position-sensing arm connected via a position resolving computer to an oscilloscope. A column of blood moving in the scanned vessel produces a Doppler shift in the reflected signal and this is indicated as a white spot on the oscilloscope

screen. Passing the probe to and fro over the vessel allows a picture to be built up on the screen which reflects the shape of the column of blood. Using pulsed Doppler imaging alone detects carotid stenosis of >25% with an overall accuracy of 72% (92); occlusion is detected with an accuracy of almost 100% (92).

2. Resistance index.

Pulsed ultrasound can also be used to measure the velocity of blood in vessels being scanned and this capability is utilised in calculation of the so-called Pourcelot index, which is a measure of carotid stenosis. Normally there is high velocity flow in the internal carotid in diastole because of the relatively low vascular resistance of the cerebral circulation. In the external carotid, as in other peripheral arteries, flow is low in diastole due to high peripheral resistance. Normally the pattern of flow in the common carotid reflects flow in the internal carotid but in the presence of internal carotid stenosis resistance increases and diastolic flow diminishes. The flow pattern in the common carotid then resembles that in the external carotid. The Pourcelot index is a measure of the resistance to diastolic flow in the common carotid and is calculated thus:

$$R = (Vp-Vd)/Vp$$

where Vp is peak velocity and Vd is diastolic velocity. A figure >0.9 is strongly indicative of a haemodynamically significant stenosis and one between 0.75 and 0.9 suggests stenosis. This test has a very low sensitivity and it is therefore useless for screening in spite of its high specificity (90).

3. Spectrum analysis.

The reflected Doppler signal from blood flowing in a major artery has a characteristic spectrum which may be displayed graphically as a frequency/time plot. A stenosis produces two principal changes in the pattern of laminar flow of fluid in a tube: an increase in velocity through the stenosed segment and turbulent flow distal to it. Both of these changes occur in blood flow through an arterial stenosis and both may be detected by analysis of changes in the Doppler shift signal spectrum. Flow through a stenosis shows an increased peak frequency compared to flow in a normal vessel because peak frequency varies directly with velocity. Examination of the signal obtained distal to the stenosis reveals spectrum broadening, an increase in the range of velocities in the vessel lumen indicative of turbulent flow. Consideration of both of these changes by an experienced technician increases the accuracy of Doppler diagnosis of significant (>25%) carotid artery stenosis to 95% (92). (For further discussion and illuatration of these techniques see chapter 5.)

Combined tests.

The accuracy of non invasive assessment may be improved by combining complementary investigations and this is the practice recommended by a number of authors. Kartchner and McRae recommend the combination of fluid oculoplethysmography and carotid phonoangiography (93). Their results demonstrate that stenosis of >40% can be detected with 90% accuracy and other reports of this combination confirm this assertion (94).

Pulsed Doppler imaging may be easily combined with waveform analysis and this is the routine screening test in the vascular laboratory in Gartnavel General Hospital, where the patients in this

thesis were studied. An extensive study of these techniques and comparisons with other approaches were carried out by another author and form the basis of a thesis (92). These results show that internal carotid stenosis of >25% is detected with an accuracy of 95%, a finding supported by others (95,96). This approach will be described in greater detail in the methods section of this thesis.

Recently Duplex scanning has become widely available and is increasing in popularity as a method for assessment of carotid disease. This investigation combines B-mode ultrasound imaging of the vessels with pulsed Doppler spectral analysis and permits more accurate grading of carotid disease. Minor irregularities of the vessel wall can be detected and repeated scans may be used to monitor changes at the carotid bifurcation. It has beeen shown that histological characteristics of carotid plaque may be accurately determined using this method, and this capability is being used to divide patients into groups at high and low risk of subsequent stroke (97). A non-homogeneous appearance of a plaque at ultrasound examination is associated with intraplaque haemorrhage, which in turn is more likely to be associated with symptomatic disease (45,98). Findings such as these are now being used as a basis for patient selection for surgery. It seems likely that as the availability of this investigation widens and experience in its use increases, it will replace existing methods of non-invasive assessment of carotid disease.

Chapter 3.

HAEMORHEOLOGY

3.1 INTRODUCTION

Rheology is the study of the flow and deformation of liquids and solids, and haemorheology is the application of this study to the constituents of blood. Interest in haemorheology is not new and at the beginning of this century a great deal of attention was focused on abnormalities of blood viscosity. There was considerable variation in the measurement of viscosity at this stage with the result that comparisons could not be made between laboratories. These difficulties were compounded by the discovery in 1915 that blood was a non-Newtonian liquid, ie, its viscosity was not constant at a given temperature. It became apparent that to yield meaningful results blood viscosity had to be measured at constant definable shear rates, and the ability to do this did not become available until the development of rotational viscometers in the 1960s. Since then these machines have come into limited clinical use and interest in haemorheology has been rekindled. This interest has been further encouraged by the limitations of vascular surgery in improving blood flow in cardiovascular disease. Before an appreciation of the place of haemorheology in modern clinical practice may be had, an understanding of some basic concepts of blood flow and viscosity is essential.

Viscosity may be considered as the resistance to flow due to internal friction between adjacent layers of a fluid. In laminar flow, adjacent layers move parallel to one another but at different velocities, and this movement of one fluid layer on another is called shearing. The velocity gradient between layers of fluid is the shear rate, given by:

$$\frac{dv}{dx}$$

This is represented diagramatically in figure 3.1. Shear rate is measured in inverse seconds (s^{-1}).

Shear stress is the force required to produce shearing and consequent flow; measured as force per unit area and expressed in Pascals (Pa).

Viscosity is defined as the ratio of the shear stress to the shear rate it produces. Thus:

$$\text{Viscosity} = \frac{\text{Shear stress}}{\text{Shear rate}} = \frac{\text{Pa}}{s^{-1}} = \text{Pa.s (Pascal seconds)}$$

In Newtonian fluids, viscosity is constant at a given temperature, ie, the ratio of shear stress to shear rate is constant, and any change in shear stress will produce a proportionate change in shear rate. The behaviour of blood does not follow this pattern; increases in shear stress produce disproportionate increases in shear rate, ie, the viscosity decreases with increasing shear rate. This property of blood is known as thixotropy and is due mainly to erythrocyte aggregation and flexibility. There is a limit to this variability of viscosity and at shear rates over 100 s^{-1} (present in most of the normal circulation) blood behaves in a Newtonian fashion. Non-Newtonian behaviour is manifest also by the existence of yield stress. Yield stress is the minimum force necessary to initiate

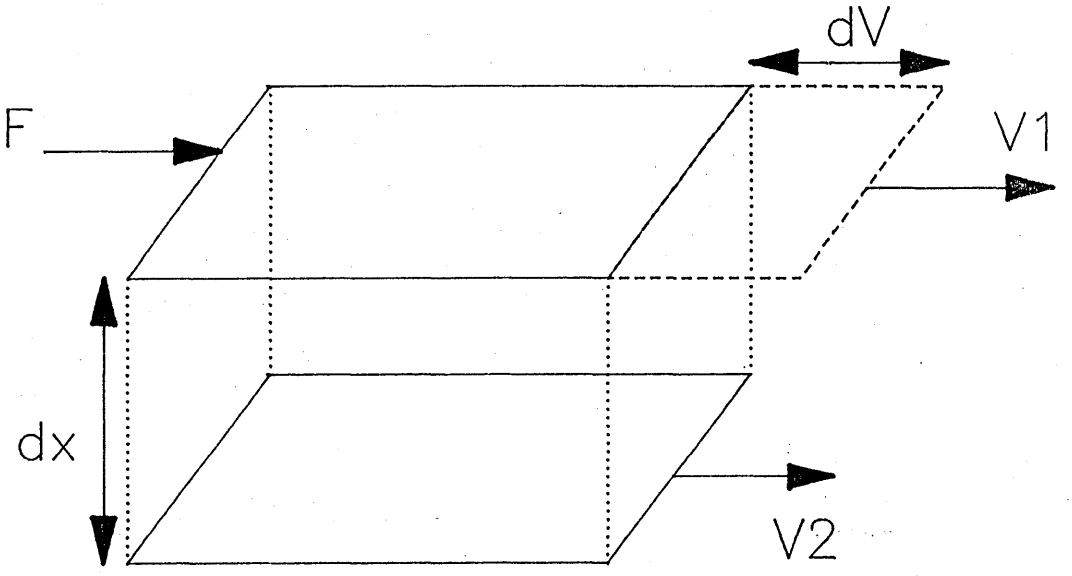

FIGURE 3.1

DIAGRAMMATIC REPRESENTATION OF SHEARING

(after Dormandy (99)).

flow; if shear stress decreases below yield stress, flow ceases. These features of the non-Newtonian properties of blood are illustrated diagrammatically in figure 3.2.

3.2 BLOOD FLOW IN LARGE VESSELS

It has been customary to consider haemorheology under the headings of flow in large (conductance) vessels (ie, greater than 100 μm in diameter), and flow in the microcirculation. Blood flow through a narrow tube was described by the French physician JLM Poiseuille in 1846 by the formula:

$$Q = \frac{\delta P \pi r^4}{8Ln}$$

where Q = blood flow, P = the pressure drop across the tube, r = the tube radius, L = the tube length and ^{n} = viscosity.

This formula was initially used to describe the flow of a Newtonian fluid and it is therefore inaccurate when describing blood flow. However, because blood behaves in a Newtonian fashion at high shear rates ($>100s^{-1}$) it allows us to discuss the major factors which determine flow in large vessels. The most important physiological influences on blood flow are exerted by perfusion pressure and vessel radius. In cerebral perfusion, blood flow is held constant over a wide range of perfusion pressure by variations in vascular tone. However, in pathological states when this autoregulatory ability is impaired or when vasodilatation is maximal, for example in the presence of ischaemia, viscosity assumes much greater importance as a determinant of flow.

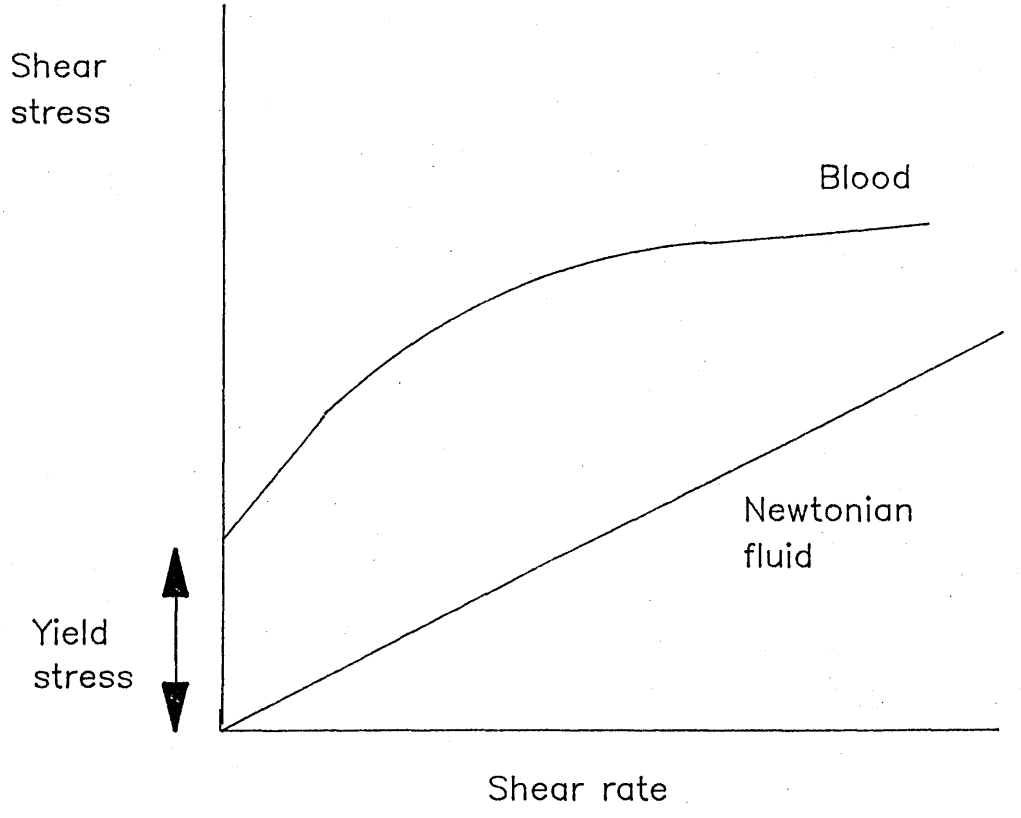

FIGURE 3.2

RELATIONSHIP BETWEEN SHEAR STRESS AND SHEAR RATE:

DIFFERENCES BETWEEN BLOOD AND NEWTONIAN FLUID

(after Dormandy (99)).

Haematocrit is the major contributor to blood viscosity at all shear rates (100,101) and, particularly above its physiological range, relatively small increases in haematocrit will produce large increases in viscosity (Figures 3.3 and 3.4). This influence of haematocrit on whole blood viscosity becomes more pronounced at low shear rates and it is an important factor in flow in smaller vessels. Increasing the haematocrit has a twofold effect on viscosity; firstly through an increase in the concentration of red cells and secondly by increasing red cell aggregation (rouleaux formation). Aggregation is reversible and is related to shear rate as well as to haematocrit. In streamlined flow in conductance vessels, shear rates are maximal close to the vessel wall and decrease towards the centre of the lumen. Red cell aggregates tend to occur in the centre of this flow where shear rates are lower. The formation of these aggregates is facilitated by the creation of intercellular bridges by protein molecules. Fibrinogen is known to be involved in formation of these linkages and any increase in its concentration leads to an increase in red cell aggregability and consequently viscosity. The non-Newtonian behaviour of blood is largely explained by these cell/protein interactions (102).

Erythrocyte deformability is another important factor in whole blood viscosity. In laminar flow in large vessels, erythrocytes deform and are oriented longitudinally (103). Any interference with this deformation will tend to increase resistance to flow. However it is in the microcirculation that erythrocyte deformability and flexibility assume their greatest importance and this will be discussed later.

Particulate constituents of blood other than red cells also contribute to viscosity. White cells are large and rigid in comparison with red cells but are normally present in such small numbers that their contribution to overall viscosity is relatively small. In

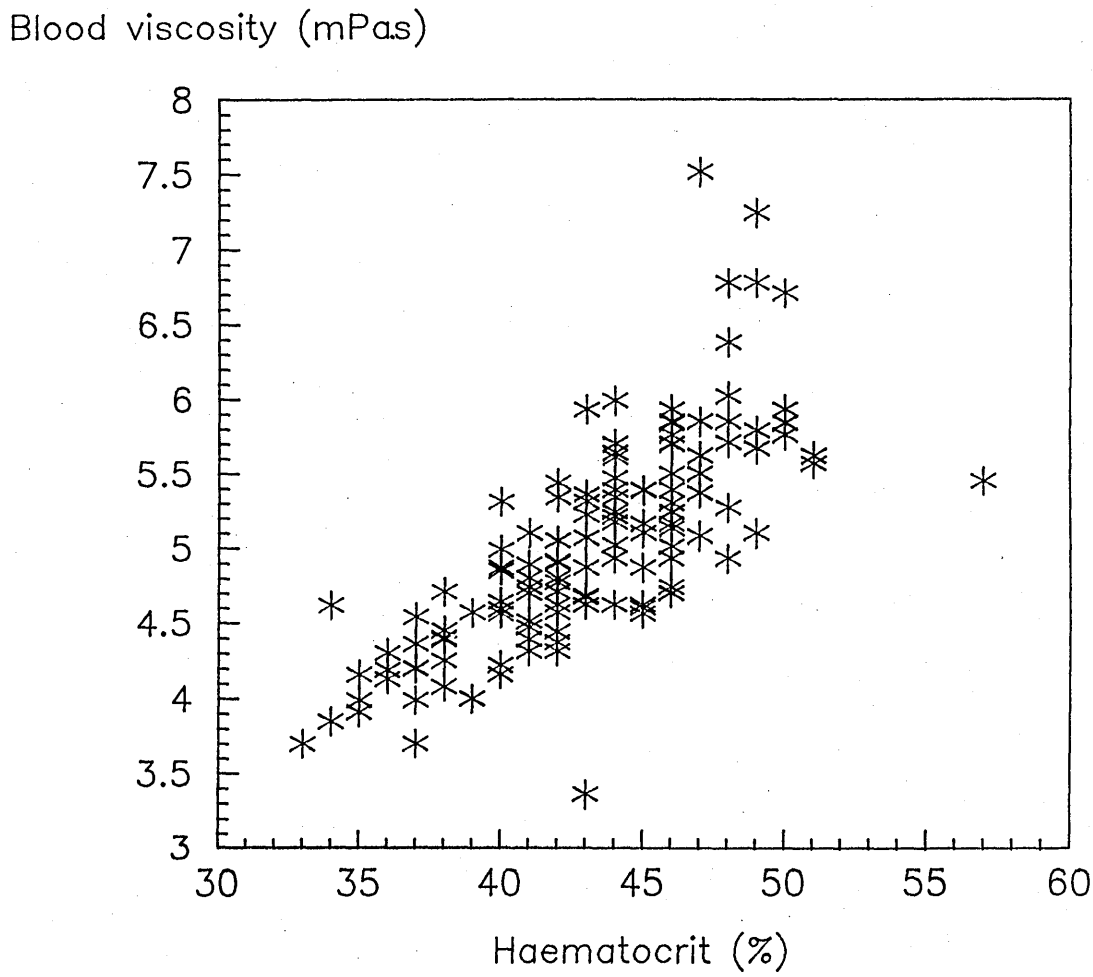

FIGURE 3.3

RELATIONSHIP BETWEEN HAEMATOCRIT AND WHOLE BLOOD VISCOSITY AT HIGH SHEAR RATE: 135 measurements in control subjects and patients with arterial disease.

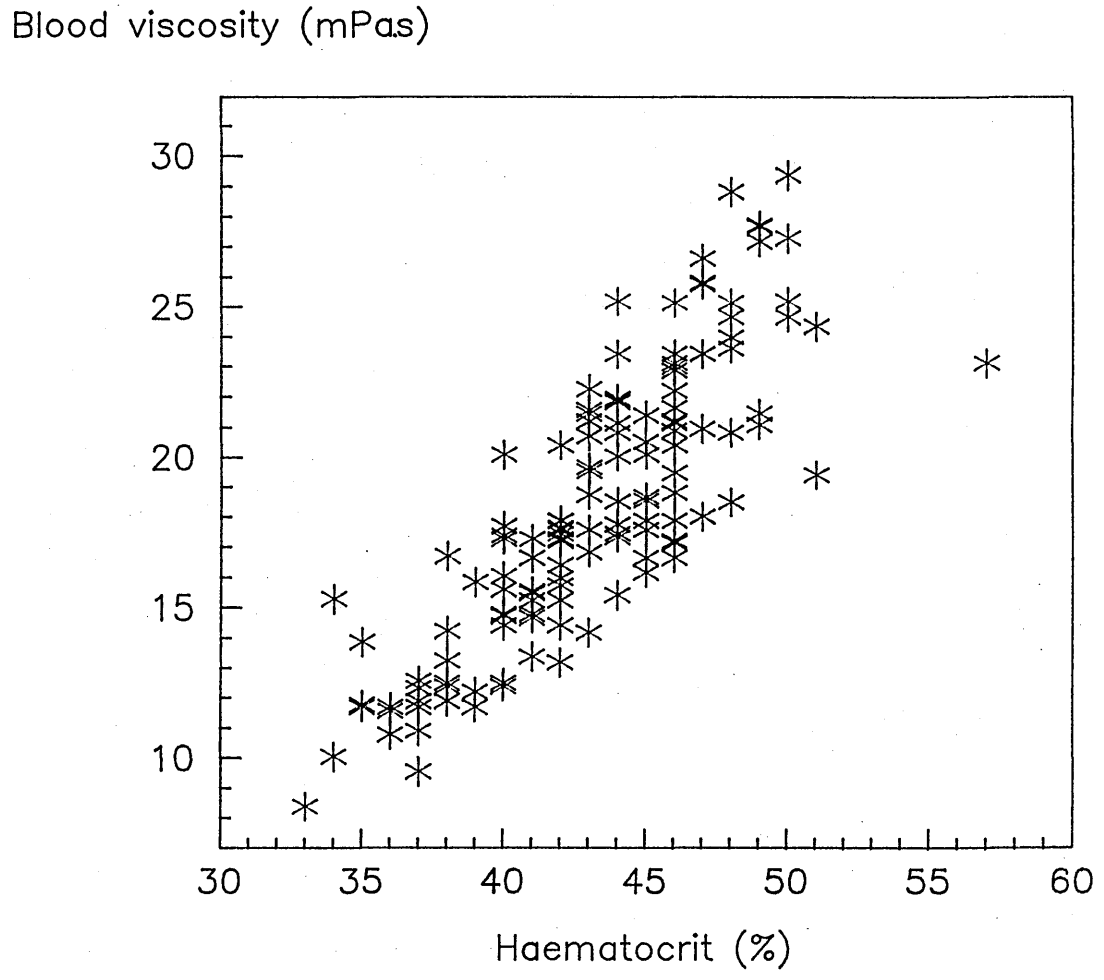

FIGURE 3.4

RELATIONSHIP BETWEEN HAEMATOCRIT AND WHOLE BLOOD VISCOSITY AT LOW SHEAR RATE: 135 measurements in control subjects and patients with arterial disease.

exceptional circumstances such as leukaemia, where large numbers of white cells are present in the peripheral blood, their presence may significantly contribute to viscosity, mainly by plugging small vessels. Platelets on the other hand, are normally present in large numbers and contribute to viscosity by aggregating. In contrast to red cell rouleaux these aggregates form irreversibly and may influence viscosity in two ways. Firstly, they may initiate intravascular coagulation and subsequent thrombus formation and secondly, aggregates may physically obstruct arterioles and capillaries. The formation of platelet aggregates is influenced by a variety of features of normal laminar flow in large vessels. Red cell aggregates occupying axial positions in the blood flow tend to displace platelets laterally to the high shear areas close to the vessel wall. This exposes platelets to high shear stress and may lead to platelet disruption and activation by release of adenosine diphosphate. The tendency of platelets to migrate towards the vessel wall is increased in the presence of enhanced erythrocyte aggregation and this in turn is increased, as we have seen, by a raised haematocrit.

The final major contributor to whole blood viscosity is plasma. Plasma viscosity is determined almost entirely by the protein concentration. The relative contributions to viscosity of the different proteins depends on their concentration, molecular size, and molecular shape. Albumin and globulin are the most abundant proteins in plasma and are responsible for about 75% of plasma viscosity. Fibrinogen makes up less than 5% of plasma protein content but is responsible for more than 20% of the measured viscosity. This is because of its very high molecular weight (340,000, about five times that of albumin) and its elongated shape. The length to diameter ratio of the fibrinogen molecule is 18.4 compared to 3.95 for albumin and

this physical property is responsible in part for the disproportionate contribution to plasma viscosity (104). As mentioned above, red cell aggregation is influenced by plasma protein concentration and fibrinogen has been shown to form bridges between red cells. The influence of fibrinogen on blood viscosity is therefore two-fold and this may explain why the fibrinogen concentration has such an apparently disproportionate influence on blood viscosity, and why factors which influence fibrinogen concentration are so important in a clinical setting.

3.3 BLOOD FLOW IN THE MICROCIRCULATION

In vessels less than 100μm in diameter blood flow is influenced by several effects which are not apparent in conductance vessels. The most important difference is an apparent reduction in haematocrit, and therefore viscosity, for which two separate mechanisms are responsible. The first, initially described in 1931, is the Fahraeus-Lindqvist phenomenon which is a reduction in the apparent viscosity of blood flowing through tubes of progressively smaller diameters (105). It is due to the dynamic reduction in haematocrit caused by an increase in velocity of red cells relative to plasma which causes red cells to move further apart. This relative change in velocity stems from axial migration of erythrocytes which consequently travel faster than peripheral plasma (103). The Fahraeus effect influences viscosity until a vessel diameter of 5-7μm is reached when a sudden increase in viscosity becomes apparent. This so-called inversion phenomenon, first observed by Dintenfass (106), occurs at a vessel size similar to the diameter of the erythrocyte and presumably

reflects the overriding importance of erythrocyte flexibility as a determinant of viscosity in vessels of this size. The second method by which haematocrit is reduced is known as the screening effect. This is a mechanical reduction in haematocrit arising from the geometry of the capillary orifice which causes plasma to be selectively channelled into it. This effect seems to be less significant than the Fahraeus effect in reducing capillary haematocrit (107).

In addition to these effects, peculiar to vessels of small diameter, viscosity in the microcirculation is affected by all of the factors which influence viscosity elsewhere in the circulation; haematocrit, red cell flexibility, red cell aggregation and plasma viscosity are the most significant. The influence of haematocrit on blood flow in the microcirculation may be less than it is in conductance vessels because of the lower haematocrit in capillary beds. It is still relevant however, not least because all of the effects described in the previous paragraph are to some extent haematocrit dependent. (107)

Erythrocyte aggregation and flexibility may have profound effects on the microcirculation, particularly in low flow states. The diameter of the vessels is such that they may be occluded by erythrocyte aggregates which can occur at low shear rates. The diameter of capillaries is often less than that of erythrocytes and capillary circulation depends on the flexibility and low internal viscosity of these cells. Increases in red cell rigidity produced by ageing, haemoglobinopathies or other disorders may have a deleterious effect on the microcirculation.

3.4 SUMMARY

Blood viscosity is the third determinant of flow after perfusion pressure and vessel radius. It is affected principally by shear rate, haematocrit, erythrocyte flexibility and aggregation, and plasma viscosity. The relative importance of each of these factors varies according to the region of the circulation under consideration.

Haemorheology is a complex subject which has only begun to be explored but the broad principles outlined in this chapter are a sufficient basis for an understanding of the studies which follow in this thesis. Some aspects require more detailed discussion and this will be done at the relevant points in the text.

Chapter 4.

CEREBROVASCULAR DISEASE AND HAEMORHEOLOGY

4.1 INTRODUCTION

Extensive investigation of patients with TIA will reveal the cause in the majority of cases. A few will have haematological disorders and other unusual explanations but the majority will be shown to have disease of the internal carotid artery or another source of emboli such as valvular heart disease. In a significant minority however, no apparent cause for the symptoms will be discovered. In De Bono's series, after investigation with ECG monitoring, cardiac ultrasound and carotid angiography, 25% of the patients came into this category (34). It was in an effort to account for the symptoms in this group that viscosity studies were instituted in our patients.

Flow of a liquid through a tube is classically described according to the Poiseuille equation as being proportional to the pressure gradient and the fourth power of the radius and inversely proportional to the tube length and the viscosity of the liquid. If cerebrovascular disease is considered in terms of the flow of blood through the cerebral vasculature then it is apparent that

abnormalities of pressure gradient and vessel radius have been implicated in its pathogenesis but that the viscosity component of this equation has been largely ignored. It is widely accepted for example that hypotension and hypertension have important cerebral manifestations, and the rôle of vessel diameter (with particular reference to the carotids) in the pathogenesis of TIA has already been mentioned (chapter 2.2). (It is important to remember however, that arterial stenosis most often contributes to symptomatic cerebral disease by acting as a source of emboli, or the site of subsequent thrombotic occlusion, and not by limiting flow.) The ease with which each of these variables is assessed may explain this state of affairs. Measurement of systemic blood pressure is easy, and accurate assessment of the carotid vasculature is now possible by virtue of many invasive and non-invasive techniques. Reproducible blood viscosity measurements on the other hand, have only relatively recently become available in a clinical situation and accurate assessment still requires expensive instruments which are not widely available. Another possible reason for the neglect of viscosity estimations is the fact that manipulation of viscosity in most cases is of no proven therapeutic benefit.

There are several reasons for believing that the study of blood viscosity (haemorheology) may be important in cerebrovascular disease:

1. A relationship between haemorheological factors and arterial disease in other sites is well established.
2. Haemorheological abnormalities have been identified in stroke patients.
3. Morbidity and mortality in stroke may be related to haemorheological factors.

4. There is a relationship between cerebral blood flow and blood viscosity.
5. Risk factors for stroke have haemorheological effects.
6. Experimental work and clinical studies suggest that reduction of viscosity may be beneficial in thrombotic stroke.

If abnormal blood viscosity is involved in the pathogenesis of cerebrovascular disease there may be important therapeutic implications. Reduction of viscosity may reduce the incidence of stroke in high risk groups, minimise the effect of stroke, or prevent recurrent stroke. The evidence supporting a link between cerebrovascular disease and haemorheology will now be reviewed under the headings outlined above.

4.2 RHEOLOGY OF ARTERIAL DISEASE

Haemorheological changes have been found both in patients with coronary artery disease and peripheral vascular disease. Several studies have demonstrated increased blood viscosity following acute myocardial infarction (108,109). Similar abnormalities exist in patients with ischaemic heart disease but without evidence of a recent myocardial infarction (110,111). Furthermore, blood viscosity has been related to the extent of coronary artery disease demonstrated at arteriography (110). The reason for increased blood viscosity in these patients is not clear. Increased haematocrit has been demonstrated by some studies (110,111), but not by others (112,113). Plasma viscosity on the other hand, is said to be increased according to most reports, and this is likely to be due to increased fibrinogen levels which have often been demonstrated in these groups of patients (110-112).

In patients with peripheral arterial disease whole blood viscosity is raised due to increases in haematocrit, fibrinogen or red cell rigidity or a combination of all three (114). A relationship has also been established between haemorheological abnormalities in patients with intermittent claudication and the need for subsequent amputation (115). If vascular reconstruction is performed on patients with lower limb ischaemia then graft survival is related to preoperative blood viscosity (116). In a recent placebo-controlled double-blind study, haemodilution sufficient to produce a reduction in viscosity was shown to be beneficial in extending the walking distance of patients with intermittent claudication (117).

4.3 RHEOLOGY AND STROKE

Several authors have found increased blood viscosity after stroke. This is mainly due to increases in haemoglobin, haematocrit and fibrinogen (118-120). Red cell deformability may be reduced, contributing to increases in viscosity but this finding has not been confirmed (121). As in patients with myocardial infarction it is uncertain if these viscosity changes precede the ischaemic event or result from it. There is a gradual reduction in viscosity among survivors as the time elapsed since the event increases but it always remains elevated compared to controls (Lowe, personal communication).

An association between elevated haematocrit and cerebral symptoms has been recognised for a long time in patients with polycythaemia. These symptoms are varied and most, like headache and vertigo, are vague and non-specific. There is also however, an increased incidence of cerebral vascular occlusive events in these patients. As observed

in chapter 2.2 polycythaemia is associated with an increased incidence of TIA. This was first reported in 1960 when Millikan described 22 patients who had both polycythaemia and transient ischaemic attacks and he concluded that a thrombotic tendency could be important in the genesis of TIA (65). In 1983 Pearce et al described three patients who presented with TIAs and who were found to have lacunar infarcts detected by CT scanning (122). All three patients had haematocrits greater than 53%. In a review in 1962 Silverstein et al observed an incidence of stroke of between 5 and 19% in polycythaemic patients (123); the vast majority of these events are due to thrombosis rather than haemorrhage and are a consequence of stasis and thrombocytosis.

This increased liability to cerebral infarction varies directly with the level of haematocrit (124) which suggests that increases in viscosity consequent on raised haematocrit may be important in cerebral infarction. An association between level of haematocrit and subsequent complications clearly has important implications for therapy; lowering the haematocrit should reduce the incidence of cerebral events in this group of patients.

Epidemiological evidence for a broader link between stroke risk and haematocrit has been provided by the Framingham study (125). Men with haemoglobin values of at least 15g/dl and women with at least 14g/dl had twice as many strokes as individuals with lower values. When the effects of smoking and hypertension, both factors associated with stroke and elevated haematocrit, were taken into account, the effect of haemoglobin level was less. It is possible that the influence of hypertension and smoking on stroke risk is partly mediated through viscosity changes. The rôle of haematocrit in the pathogenesis of cerebral infarction was further studied by Toghi and his colleagues in 432 post mortem examinations (126). They

demonstrated that the risk of cerebral infarction was increased in patients with a high haematocrit and this risk increased sharply when haematocrit values exceeded 45%. Perhaps not surprisingly they also observed that this increased risk is more obvious in patients with severe cerebral atherosclerosis than in those with mild arterial disease.

4.4 MORBIDITY AND MORTALITY

Mortality after acute stroke has been related to abnormal blood viscosity. Both raised haematocrit and fibrinogen are associated with increased hospital mortality (120,127). Recovery from stroke is thought to depend in part on rapid development of collateral flow and this may be impaired if blood viscosity is increased. Following carotid occlusion in gerbils, the size of cerebral infarct produced increases with the haematocrit (128). Similarly, in patients with carotid occlusion, Harrison found that the size of infarct measured by CT scan was related to the haematocrit (129). It is accepted that at the margins of a cerebral infarct, autoregulation is impaired and dynamic control of vasodilatation and vasoconstriction is lost (130). Under these circumstances blood flow is determined by perfusion pressure and viscosity. If a major vascular occlusion is the underlying pathology, perfusion pressure will be reduced and viscosity may be the most important determinant of flow. Under these circumstances increased viscosity will reduce perfusion and may lead to an extension of the volume of infarction.

4.5 CEREBRAL BLOOD FLOW

One explanation of the relationship between high haematocrit and cerebrovascular disease may lie with the decreased cerebral blood flow (CBF) which occurs in patients with raised packed cell volumes. Sluggish flow leading to stasis and thrombosis is an appealing concept. The cause of the decreased CBF in patients with a high haematocrit is the subject of some controversy. Venesection produces an increase in CBF and early studies concluded that this was at least partly due to the decrease in whole blood viscosity which follows venesection (66,67). However, arterial oxygen content is also reduced by venesection and cerebral autoregulation will cause CBF to increase to maintain cerebral oxygen transport. It was initially thought that this change in oxygen carriage was not sufficient to explain fully the observed changes in CBF and viscosity alterations were also relevant.

Later clinical studies cast doubt on this conclusion. In a study in 59 paraproteinaemic patients, Brown found a significant correlation between CBF and arterial oxygen content but no correlation between CBF and viscosity (131). The same author, in another study, observed arterial oxygen content and whole blood viscosity in relation to CBF in 54 subjects with a wide range of haematocrits (132). Again arterial oxygen content appeared to be the main determinant of CBF. Blood viscosity had no significant influence on CBF once arterial oxygen content had been taken into account. In contrast, recent animal studies to assess the relative contributions of viscosity and arterial oxygen content to the control of CBF have not substantiated these clinical observations. Hudak and Muizelaar, in lambs and gerbils respectively, have independently shown that both arterial oxygen content and blood viscosity contribute to the control of CBF

(133,134). On balance it seems that both viscosity and arterial oxygen content regulate CBF to some extent but that in man the latter is by far the more important.

In any event, observations on the control of CBF in subjects with normal vasculature may not be entirely relevant in an elderly population with a high incidence of cerebrovascular disease. There are limits to cerebral autoregulation and, when these are exceeded, blood flow passively follows changes in perfusion pressure and blood viscosity. The importance of viscosity in determining flow may then be increased. Grotta in 1982 studied 53 patients and controls, and demonstrated correlation between blood viscosity and CBF (101). Both haematocrit and fibrinogen levels correlated inversely with CBF when examined individually, but the association with CBF was strongest when they were considered together.

4.6 RISK FACTORS

The relationship between high haematocrit, whether pathological or "high normal", and stroke risk has been discussed (chapter 3.3). Other factors which influence blood viscosity have also been shown to be risk factors for stroke. Wilhelmsen et al, in a prospective study of 792 men, found that raised fibrinogen and blood pressure were positively correlated with subsequent stroke (135). Although the association between fibrinogen and stroke was weaker when blood pressure, serum cholesterol and smoking were taken into account, it remained significant. Since hypertension and smoking are both

associated with a raised plasma fibrinogen (136,137), it is possible once again that the association between these risk factors and stroke is mediated via viscosity changes.

4.7 HAEMORHEOLOGICAL THERAPY IN STROKE

The observation that increased blood viscosity produced by an elevated haematocrit increases the volume of infarcts following vascular occlusion suggests that viscosity changes in the opposite direction may be beneficial (128,129). In patients with an adequate collateral circulation an increase in cerebral blood flow following a reduction in haematocrit might be expected to improve perfusion at the margins of an infarct sufficiently to limit the size of that infarct. In an uncontrolled study of hypervolaemic haemodilution in nine stroke patients, Wood and Fleischer (138) observed neurological improvement in eight. In this study mean haematocrit was reduced from 41% to 32%, mean arterial pressure from 101 to 94mmHg and central venous pressure raised from 4 to 12cmH_2O. Despite the reduction in haematocrit it was calculated from previous experimental work that oxygen delivery (blood oxygen content x flow) was increased, particularly in ischaemic tissue, and this constitutes the theoretical basis for the efficacy of haemodilution. Further work by the same group, this time combining venesection with intravenous infusion to produce isovolaemic haemodilution, demonstrated encouraging quantitative EEG changes in 11 stroke patients (139).

Recovery from stroke is immensely variable and clearly assessment of any therapy properly requires a randomised study. Strand et al carried out such a study on 102 stroke patients of whom 52 received

isovolaemic haemodilution therapy in the form of venesection of 250-650ml and synchronous infusion of Dextran 40, while the remaining 50 acted as controls (140). Assessment of both groups at three months following the ischaemic event revealed identical mortality rates. Among the survivors however, 13% of the treatment group were still in hospital as opposed to 39% of the controls and the figures for those unable to walk were 8% and 31% respectively. In view of these encouraging results further randomised studies are under way.

4.8 CONCLUSION

It appears from evidence cited here that there is a substantial link between blood viscosity and its determinants, and cerebrovascular disease. In some circumstances, primary proliferative polycythaemia for example, abnormal viscosity is directly implicated in cerebrovascular events, while in others, eg, elevated plasma fibrinogen, viscosity changes and stroke may be linked simply through common aetiological factors. Further study of the rôle of blood viscosity in cerebrovascular disease is warranted, firstly because it may increase our understanding of the condition and its pathophysiology, and secondly because manipulation of viscosity may be beneficial, not only in established stroke (see above), but in stroke prevention.

Chapter 5.

METHODS AND PATIENTS

5.1 INTRODUCTION

Several techniques are common to some of the studies described in this thesis and it is appropriate at this stage to describe these in detail. In recognition of the fact that some of the investigations are not widely available and are familiar only to limited numbers of specialists, a discussion of the fundamental principles involved and their place in general medical practice is included. The recruitment of patients and control subjects for the various studies will also be discussed. The chapter will conclude with a description of the statistical methods employed in analysis of the various data obtained in the studies.

5.2 PLASMA VISCOSITY

A. Principles.

Plasma may be considered simply as a plasma protein solution for purposes of viscosity measurement. Other constituents of plasma such as urea and glucose, contribute very little to viscosity and even extreme changes in the levels of these solutes produce no measurable changes in viscosity (89). Although plasma viscosity correlates with total protein concentration, this correlation is poor due to the variable effects of different proteins on viscosity. The contribution

of any particular protein to total viscosity depends not only on its concentration but its molecular size and configuration. Fibrinogen, despite its relatively low concentration, has a disproportionately large effect on viscosity because of its molecular shape. The long narrow fibrinogen molecule disrupts laminar flow much more effectively than the more compact albumin.

Unlike whole blood, plasma is a Newtonian fluid and its behaviour will conform to the relationship described by the Poiseuille equation:

$$Q = \frac{\delta P \pi r^4}{8Ln}$$

The viscosity of plasma is independent of shear rate.

Plasma viscosity can be effectively determined in capillary viscometers in which the rate of flow along a tube of known radius and length under a constant head of pressure can be measured, and viscosity calculated from this. Because viscosity varies with temperature it must be measured at a fixed known temperature to allow meaningful comparison of results (see below).

Plasma viscosity remains remarkably constant in the absence of extreme physiological insult. Overhydration, dehydration, and strenuous exercise may produce measurable changes and should be avoided prior to specimen collection. There is evidence for circadian influence on plasma protein concentration and the timing of sampling should be decided bearing this in mind.

B. Plasma Viscometers

Several methods are available for satisfactory measurement of plasma viscosity: capillary viscometers, rotational viscometers, and falling ball viscometers have all been recommended. The capillary viscometer developed by Harkness has been available commercially for

some years, is widely used and is now the recommended method (141,142). It is an accurate machine, is relatively simple to use and large numbers of samples may be processed rapidly. It has been used for several years in the Department of Medicine laboratory in the Royal Infirmary, Glasgow where the measurements for this thesis were carried out.

The Coulter Harkness viscometer (Coulter Electronics Ltd., Harpenden, Hertfordshire, England) consists of the following components:

1. Capillary section: precision bore glass tubing of 0.38mm in diameter.
2. Mercury reservoir: to provide a constant head of pressure. This section incorporates electrodes to operate the electronic timer.
3. Connecting tubes and reservoir primed with 3.6% saline, the density of which is close to the mean density of plasma.
4. Electric pump.
5. Electronic timer.
6. Water bath.

The viscometer is pictured in figure 5.1 and represented diagrammatically in figure 5.2. The plasma sample is added to the cup and is then drawn along the capillary under a constant head of pressure provided by the mercury column. The electronic timer is switched on and then off as the mercury meniscus passes the two electrodes in the horizontal limb of the mercury filled section of the tubing. The timer measures in steps of 0.02 seconds and the recorded time is displayed until the timer is manually reset. Waste samples are drawn off by the pump attached to the reservoir on the left of the

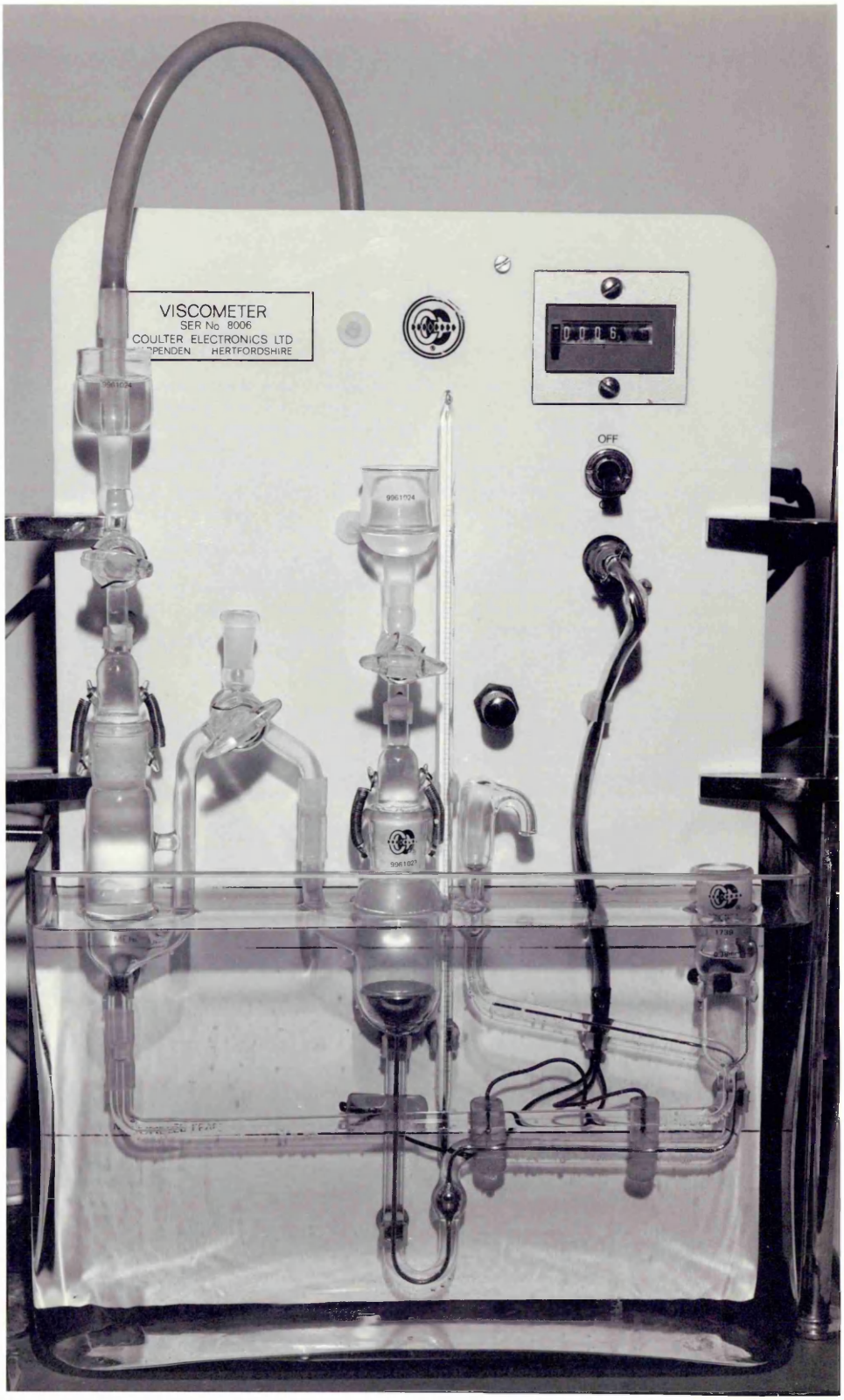

FIGURE 5.1

COULTER-HARKNESS CAPILLARY VISCOMETER.

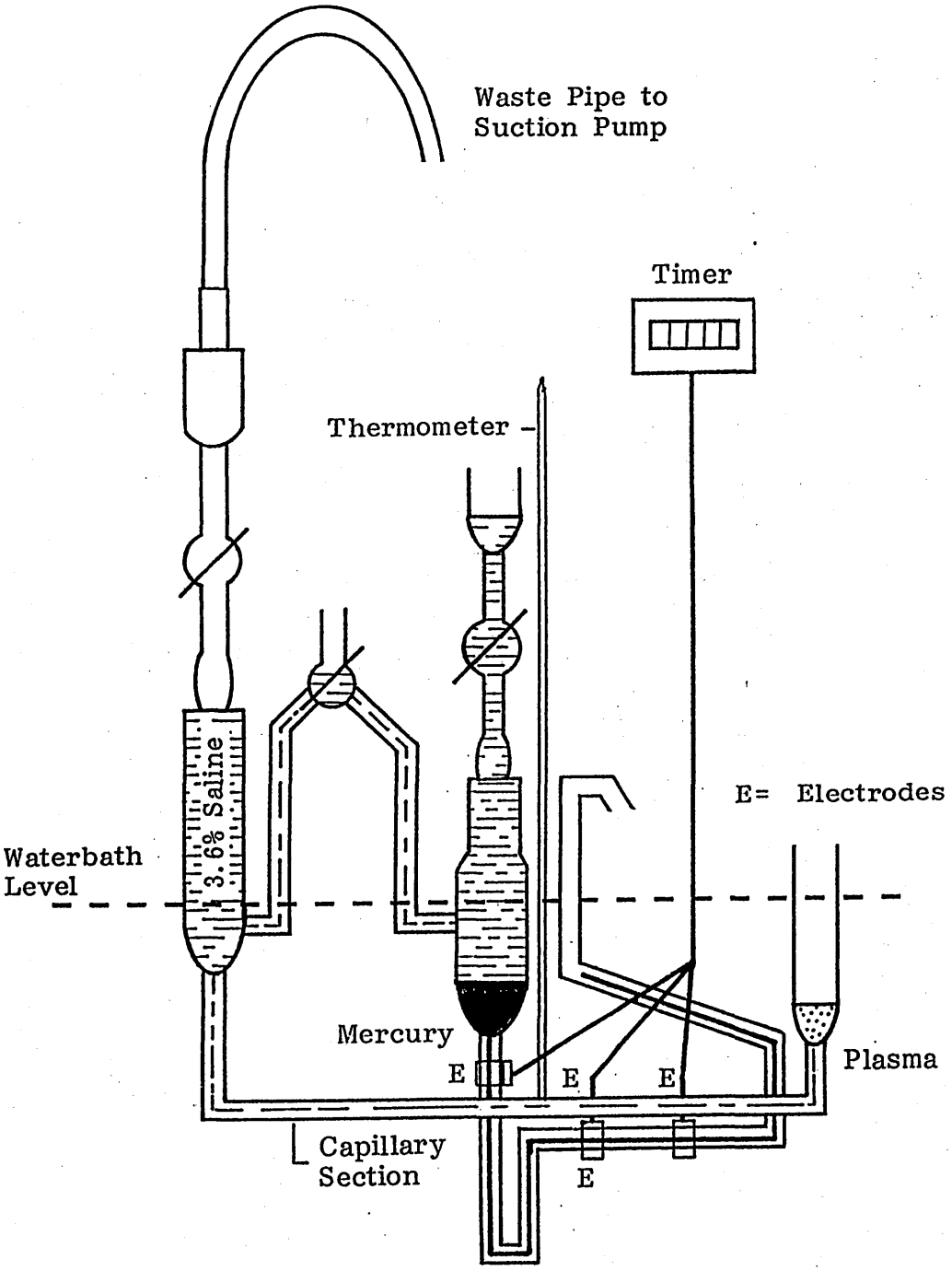

FIGURE 5.2

DIAGRAMMATIC REPRESENTATION OF COULTER-HARKNESS VISCOMETER

(compare with figure 5.1).

instrument. The entire measuring section of the instrument is immersed in the water bath at a constant temperature (initially 25°C and later 37°C, in this series of studies)

C. Measurement

The electronic timer measures the time taken for 0.2ml of mercury to pass between the electrodes; an identical volume of the test fluid is drawn along the capillary in the same interval. The viscosity of the test fluid can then be calculated as follows:

$$\text{Viscosity of test fluid} = \frac{\text{Viscosity of standard} \times \text{time flow of test}}{\text{Time flow of standard}}$$

In this case the standard fluid is 3.6% saline, which has a density similar to plasma. By adjusting the head of pressure the time flow in seconds of the standard solution may be altered to become equal to ten times the viscosity in mPa.s. The calculation then becomes:

$$\text{Viscosity of test fluid} = 0.1 \times \text{time of flow of test fluid.}$$

Each sample is measured twice (only 0.5 ml. is required each time) and the mean taken as the result.

The reference values for plasma viscosity at 37°C are 1.16-1.37 mPa.s in adults, until late middle age when increasing plasma fibrinogen causes a gradual rise (142).

D. Collection and preparation of plasma sample

Venous blood was collected via an antecubital vein without stasis (to avoid increase in plasma protein concentration) and transferred to a standard full blood count container. No anticoagulant is free from effect on plasma viscosity but potassium edetate at concentrations provided in these bottles by addition of exactly 5ml of blood has least effect (142).

Separation of plasma (by centrifugation at 3,000 g for 10 minutes) was carried out within four hours. Prolonged contact between plasma and red cells produces increased plasma viscosity with shift of water into the intracellular compartment and results are therefore influenced by the delay between sampling and centrifugation. All samples in this series were treated identically and this delay was always between three and four hours and should have negligible influence on the results. Once plasma has been separated from the cellular blood components, it may be stored in a stoppered tube at room temperature for up to a week without change in viscosity. In the course of these studies samples were stored like this for up to one week to allow processing to be carried out in batches.

E. Temperature correction

The relationship between plasma viscosity and temperature is virtually linear between 20° C and 40° C, and measurement may be made anywhere within this range and the result corrected for temperature using a conversion factor (143). The International Committee for Standardisation in Haematology has recently published guidelines for the measurement of plasma viscosity and the preferred method is to carry out measurements at 37°C which obviates the need for correction

(142). Initially measurements for studies in this thesis were made at 25°C but later, in the light of these recommendations, 37°C was the chosen temperature. The first few results obtained at 25°C were corrected to 37° C by use of the conversion factor (1.288) (143).

5.3 WHOLE BLOOD VISCOSITY

A. Principles.

The measurement of whole blood viscosity, compared to the relatively simple techniques for measuring plasma viscosity, is a complex procedure fraught with potential sources of error. Most difficulties arise because blood is non-Newtonian in behaviour, in that its viscosity varies with shear rate. This discovery, made early this century, meant that interest in the clinical relevance of blood viscosity was largely suspended until the 1960s when rotational viscometers became available commercially. These instruments allow viscosity measurements to be made at constant definable shear rates and give reproducible results which permit comparisons between different centres of study.

Viscosity is defined as the ratio of shear stress to shear rate (chapter 3) and in Newtonian liquids this is constant. To determine viscosity in a non-Newtonian liquid it is necessary to maintain either the shear stress or the shear rate at a constant level and measure the other. Viscosity is then calculated using the ratio and expressed as viscosity at that shear rate. In addition to shear rate dependence, whole blood viscosity is also temperature dependent and measurements must be carried out at a fixed temperature, usually 37°C.

The principle of most rotational viscometers is that when a thin layer of blood is sheared between two surfaces by rotating one of them, the torque produced is proportional to the resulting shear stress. Viscosity is then calculated thus:

$$\text{Viscosity} = \frac{\text{Shear stress}}{\text{Shear rate}}$$

Several different viscometers are commercially available for clinical use. The geometry of the surfaces between which the blood is sheared varies from instrument to instrument, as does the method for producing shear and measuring shear stress. In some instruments the shearing is produced, and the resultant shear stress measured, by the same component, while in others the shearing is produced by one surface and the torque measured at the other. The instrument used for the studies presented in this thesis belongs in the latter category and is described below.

B. Viscometry.

The viscometer used in this study was the Contraves L.S. 30 viscometer (Contraves Industrial Products Ltd., Times House, Ruislip, Middlesex.)(Figures 5.3 and 5.4). It is well established in clinical research and gives accurate reproducible results at high and low shear rates (99). It is a co-axial cylinder (Couette) viscometer, and blood is sheared between the surfaces of the cup containing the sample and the bob suspended in it. The shear stress is applied by the cup and the resulting torque measured by the bob. The diameters of both bob and cup are large compared to the thickness of the layer of blood trapped between them, and the shear rate throughout the sample is consequently fairly constant.

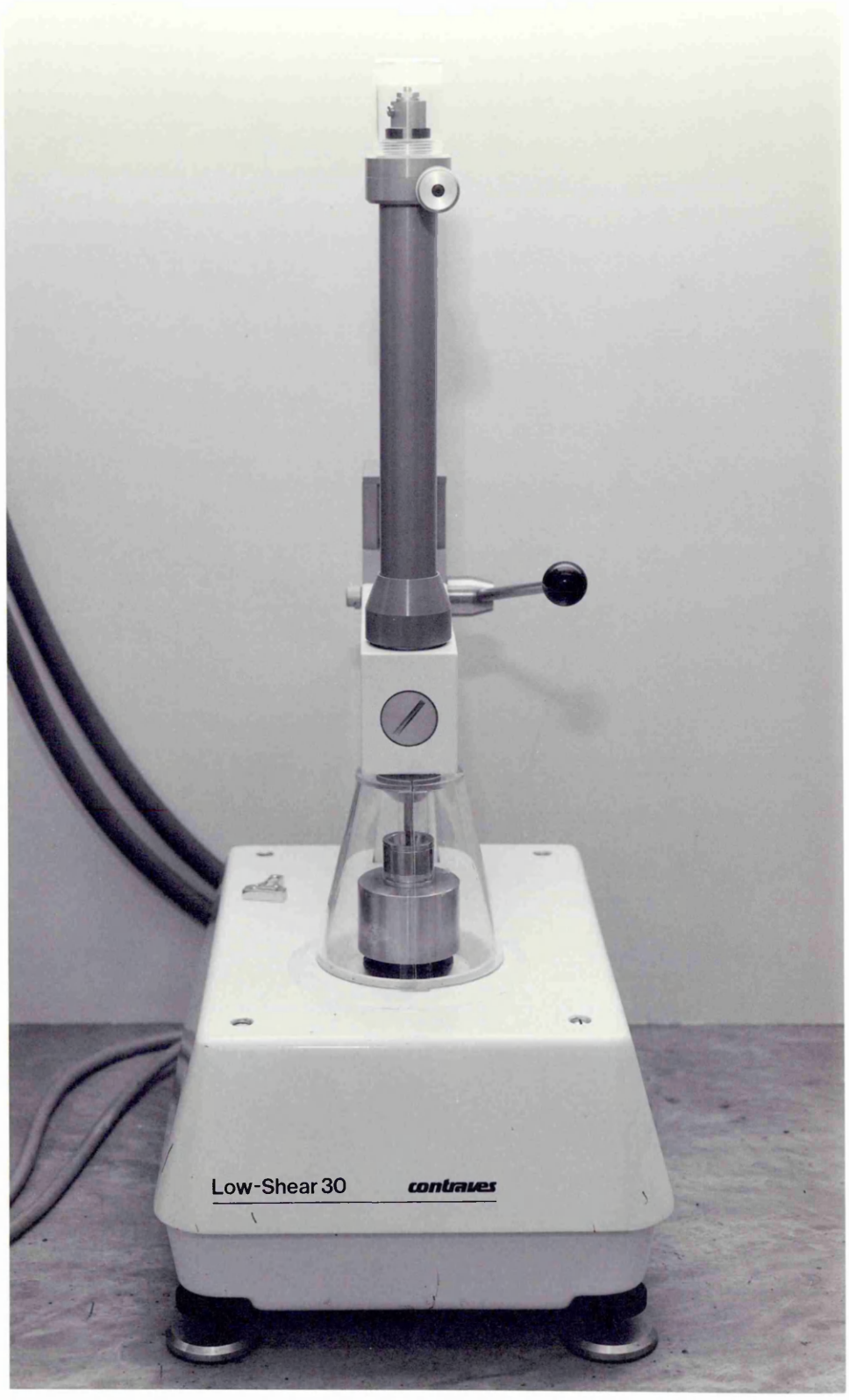

FIGURE 5.3

CONTRAVES LS 30 VISCOMETER.

FIGURE 5.4

RHEOSCAN 20 PROGRAMMING UNIT.

As the cup rotates, torque is applied to the bob via the sample and this causes deflection of the tension wire on which the bob is suspended. This deflection is opposed by torque exerted on the upper end of the wire by an electromagnet whose current is controlled by an automatic feedback mechanism which operates via a mirror attached to the upper end of the wire. Deflection of the wire and mirror is detected by a photoelectric system which is responsible, via a differential amplifier, for the variable current to the electromagnet. This current is proportional to the torque exerted on the bob by the sample and is displayed graphically against time using an XY/time recorder (Bryans Southern Instruments Ltd., Wilton Lane, Mitcham, Surrey, England.) The viscometer is represented diagrammatically in figure 5.5.

The environment of the viscometer is a constant source of possible measurement error. Interference, with low shear measurements in particular, may arise from air currents around the bob, vibration through the surface on which the viscometer stands and air/sample surface tension artefacts. In order to minimise these effects the bob and cup are sheltered by a perspex draught shield, the viscometer is mounted on a concrete bench and a guard ring is fitted over the top of the sample. The temperature at which measurements are made is determined by a thermostatically controlled water jacket which surrounds the cup.

C. Measurement.

The variation of blood viscosity with shear rate makes it obligatory to measure viscosity at more than one rate to obtain an accurate overall assessment of any sample's characteristics. The International Committee for Standardisation in Haematology has

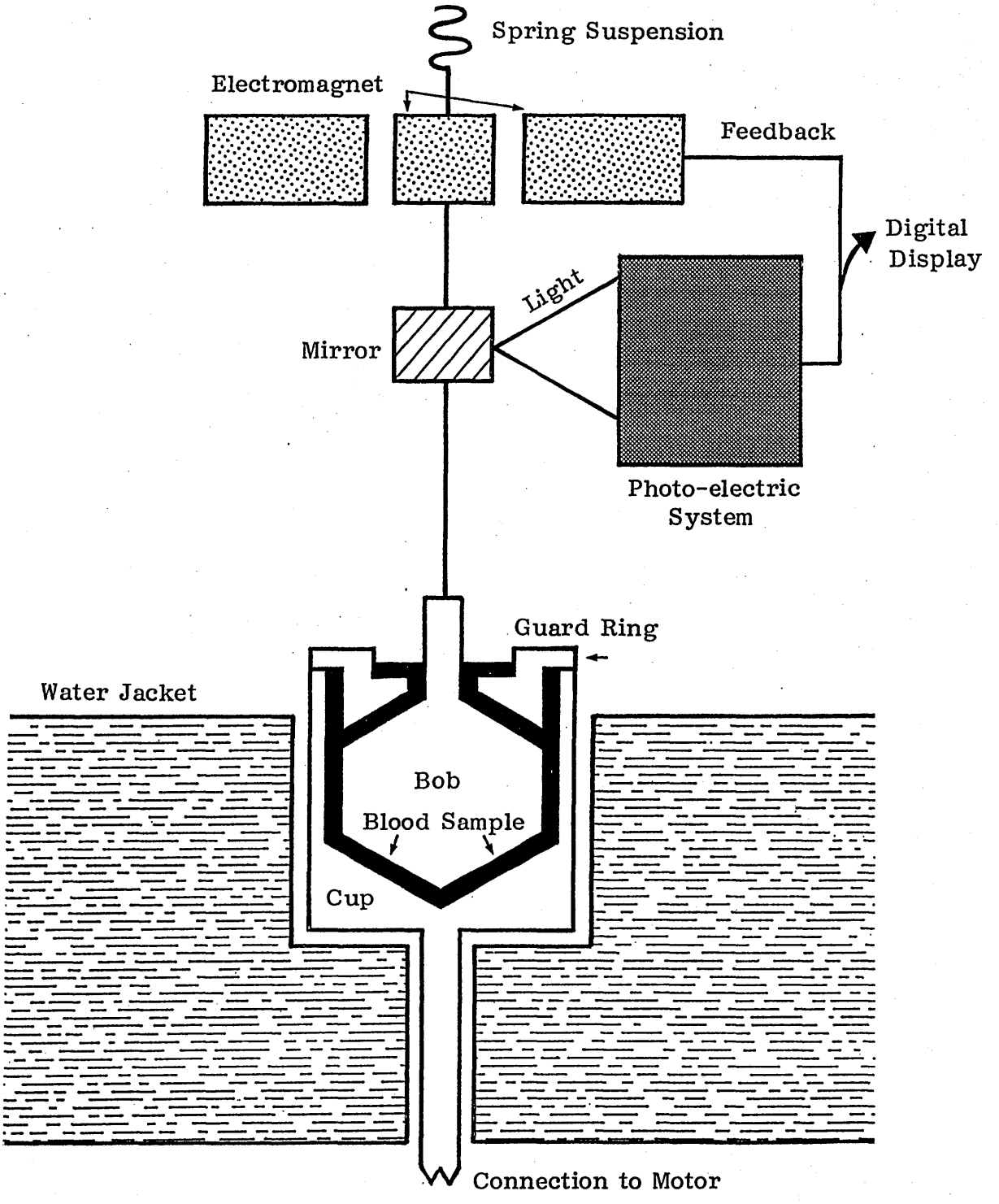

FIGURE 5.5

DIAGRAMMATIC REPRESENTATION OF CONTRAVES LS 30 VISCOMETER.

suggested that shear rates of $1s^{-1}$ and $100s^{-1}$ are appropriate (144). At shear rates much below $1s^{-1}$ (ie, very low shear rates) phase separation leads to inaccurate, artificially low readings, while at rates in excess of $100s^{-1}$ the shear dependence of blood viscosity disappears and it assumes near Newtonian characteristics as erythrocyte aggregates disperse and cells deform. For practical purposes the viscosity studies in this thesis were performed at $94.5s^{-1}$ and $0.945s^{-1}$ which are close to the recommended rates.

There is a delay after addition of the sample to the cup, before the shear programme is run, to allow bubbles to be removed from the sample and to permit the sample to reach the appropriate temperature. In an attempt to minimise the effects of phase separation which may affect low shear measurements the high shear programme is run first so that the sample is remixed just before the low shear programme.

D. Haematocrit correction.

The contribution of haematocrit to whole blood viscosity has already been emphasised. In order to assess the contribution of other factors it is necessary to correct viscosity measurements to a standard haematocrit if potentially significant changes are not to be obscured. Minor but perhaps clinically meaningful alterations in red cell rigidity or aggregation may easily be concealed by relatively small variations in haematocrit, such is its influence on viscosity. Furthermore it is apparent that haematocrit is not constant throughout the circulation and its influence in vivo will therefore vary.

Correction for haematocrit may be accomplished in three ways: each sample may be reconstituted to a standard haematocrit by the removal of red cells or plasma (99); a reference curve of "normal"

samples of differing haematocrits may be constructed and test samples compared to it (144); or a regression equation such as that derived by Matrai using haematocrit, plasma viscosity and apparent whole blood viscosity may be utilised (145). In practice the first option has a number of drawbacks, the most important of which are that it is time-consuming and potentially very inaccurate. In this thesis the regression equation below was used:

$$\text{Corrected viscosity} = \text{Exp}(\text{Log}_e(PV)+(45/Hct)x\text{Log}_e(BV/PV))$$

where: PV = Plasma viscosity
Hct = Haematocrit
BV = Apparent blood viscosity (145).

Haematocrit was measured by the microhaematocrit method using the Hawksley centrifuge. This method is accurate, rapid, uses a small volume of blood and is inexpensive. Samples were spun at 15,000g for five minutes and were measured in duplicate; no allowance was made for plasma trapping between cells (144).

5.4 FIBRINOGEN

Several methods are available for the measurement of plasma fibrinogen. The most popular, and the one which has been used for all samples in this thesis, was initially described by von Clauss. The principle on which this test depends is that the fibrinogen concentration of a plasma sample is inversely proportional to the time taken for clot to form after the addition of thrombin to the sample.

The clotting time of the test sample is compared to a previously constructed standard curve of fibrinogen concentration against clotting time and the fibrinogen concentration of the sample derived (146).

Plasma samples were prepared by the addition of 4.5ml of venous blood, to 0.5 ml 3.2% trisodium citrate and centrifuging this sample at 3,000g for 10 minutes; the supernatant plasma was then removed to a dry container.

Following dilution of this platelet-poor plasma sample 1:10 with buffer the test sample was introduced to the semi-automated Coag-A-Mate (General Diagnostics, Miami, Florida, USA) which obviates the need for determining the precise moment of thrombus formation by eye.

Samples may be analysed in batches, if desired, by storing separated plasma at -20°C; previous studies have demonstrated no significant change in measurements repeated after storage for one week in this fashion (147).

5.5 BLOOD SAMPLING

A standard routine when sampling blood for viscosity studies is desirable. At each of the stages of withdrawing and preparing a sample variations in technique can influence subsequent results. It is particularly important in controlled studies that all subjects are treated identically.

Plasma viscosity, haematocrit and whole blood viscosity exhibit small circadian changes and, if possible, all samples should be taken at the same time of day (142,144). Changes in these variables may also

be induced by extreme physical exercise and alterations in posture (142,144). Venous stasis prior to withdrawal of blood samples produces plasma protein changes and alterations in plasma viscosity. Too rapid withdrawal of blood, or use of a narrow guage needle will damage the cellular elements and produce errors. No anticoagulant is free from effect on haemorheological measurements but potassium edetate has minimal effects and is recommended (144). Dry anticoagulant will prevent dilution effects. The length of delay between sampling and analysis is critical, and if prolonged delay is unavoidable the method of storage of samples is important. In ideal circumstances blood viscosity should be estimated within four hours of sampling, and plasma for viscosity measurements should be separated from the cellular elements within the same time period (144). Plasma, once separated, may be stored in stoppered tubes to prevent evaporation,for up to one week at room temperature, without significant change in viscosity (104).

Mindful of these possible sources of error, all patients and control subjects studied in this thesis were treated identically. Samples were taken in the early afternoon, without fasting, and analysed, or separated for later analysis in the case of plasma viscosity, within four hours. Blood was withdrawn using a plastic 20ml syringe and a 21 guage needle from an antecubital vein. All subjects were seated at the time of sampling and blood was removed without stasis. If the application of a tourniquet was necessary for venepuncture it was removed for at least five seconds before withdrawing blood. Standard full blood count containers with dry EDTA were used to collect whole blood and plasma viscosity samples. The quantity of EDTA in these containers is sufficient to provide ideal concentrations of anticoagulant (3.4 - 4.8mmol/l) after addition of

exactly 5ml of blood (144). The tubes were inverted ten times, rather than shaken, to ensure thorough mixing but prevent bubble formation and cellular damage. Fibrinogen estimations were carried out on citrated samples as previously described (chapter 5.4). Microhaematocrit measurements were performed on a well mixed aliquot from the sample used for the determination of whole blood viscosity.

5.6 DOPPLER CAROTID SCANNING

As observed in chapter 2.4, a large proportion of patients with symptoms of carotid distribution TIA have disease of the ipsilateral extracranial carotid artery, and the presence and severity of such disease is directly related to the risk of subsequent stroke (46,85). Surgical correction of stenosis by carotid endarterectomy in selected patients, by experienced surgeons, reduces the incidence of stroke and recurrent TIA (148-150) although the superiority of surgical over medical management of these patients has not yet been demonstrated in a controlled trial. Because surgical management is so widely practised (over 100,000 carotid endarterectomies are now performed each year in the USA (151)), the detection of patients with disease amenable to surgery has assumed great importance in recent years. Arteriography is the traditional method of investigation, as well as being the yardstick for other methods, and few surgeons will undertake endarterectomy without first reviewing an arteriogram. However, whether performed by intra-arterial injection or via the intravenous route with the aid of digital subtraction, arteriography is an invasive procedure not without risk. Although morbidity has lessened in recent years it is still measurable, and consequently this

investigation is unsuitable for use as a screening procedure, particularly in view of the large percentage of patients who will not eventually require surgery.

Most vascular surgeons now use non-invasive assessment to evaluate the carotid circulation, and submit only selected individuals to angiography. In the UK, more than 80% of the surgeons who regularly perform carotid surgery use ultrasound as a preliminary investigation (152) and this is the routine in the vascular laboratory in Gartnavel General Hospital, where the studies for this thesis were carried out. Previous evaluation of the screening in this laboratory, using arteriography as the standard, has demonstrated an overall accuracy of 96% in the detection of carotid stenosis of 25% or more (92), and this compares favourably with reports from other centres on the use of either similar or different methods (93-95).

A detailed discussion of the physics and mathematics of the Doppler effect and its measurement is outwith the scope of this thesis, but an understanding of the broad principles underlying the use of Doppler ultrasonography in the assessment of the carotid circulation is desirable.

The Doppler principle states that the frequency of sound detected by a receiver will differ from the frequency emitted by the source if the distance between source and receiver changes during emission of the sound. The frequency detected by the receiver will be higher if the source is moving closer and lower if it is moving away. If we now consider a receiver and a source situated side by side and sound being reflected from a target, which therefore effectively becomes the source, then movement of the target relative to the source/receiver will effect similar frequency changes in the reflected sound. The difference between the emitted and the received frequencies is the

Doppler shift, and this may be detected by a demodulator, which compares the waveforms of the initial and reflected sounds and generates the Doppler difference waveform in response. Appropriate analysis of the Doppler difference waveform yields information on the velocity of the target. These effects have been described in relation to a system in which the target is a single object moving in the axis of an ultrasound beam which is continuous, but they are equally applicable, if more complex, when applied to multiple objects, such as the cellular elements of the blood, moving obliquely in the axis of the beam.

With continuous-wave Doppler ultrasound the detection of a Doppler shift in the beam gives no information on the distance between the object producing the shift, and the source/receiver. This information is clearly of some importance if particular blood vessels are to be examined without interference from overlying or more deeply placed vascular structures. This handicap is overcome by combining Doppler ultrasound with the pulse-echo principle. If the reflected signal is sampled after a defined time interval then knowledge of the velocity of sound through tissue permits calculation of the distance from the transducer face to the object being scanned. This process is facilitated by producing pulses of ultrasound rather than a continuous wave, and these may be coded by varying their amplitude. Thus a pulse-Doppler can detect Doppler shift in a specific volume of tissue, the sample volume, which is defined by the timing of the sampling and the beam width.

Moving from general to specific considerations, it is appropriate at this point to describe the instruments currently used in the vascular laboratory in Gartnavel General Hospital. According to the classification described in chapter 2.4, Doppler screening in this

laboratory is a combined test; the first part is Doppler imaging and the second, spectrum analysis. Imaging is performed using the Hokanson P2 Ultrasonic Arteriograph supplied by D.E.Hokanson, Issaquah, Washington, USA. This instrument essentially comprises an ultrasonic probe attached to a position-sensing arm, a position computer and an oscilloscope screen. As the probe is moved across the skin overlying the vessl to be scanned, points at which Doppler shifts are detected are marked and stored on the oscilloscope screeen as bright spots. A two dimensional "map" of the vessel is gradually built up on the screen as the probe is passed to and fro over the vessel. Examples of normal and abnormal carotid images are shown in figures 5.6, 5.7 and 5.8. Imaging of the vessel in this fashion is a sensitive test for cariotid occlusion but is much less useful in the detection of stenosis. In order to identify stenoses more information must be extracted from the Doppler shift signal by spectrum analysis.

Spectrum analysis of the Doppler shift signal may be used to produce a frequency/time plot of the signal. This is performed by a spectrum analyser which decodes the shift signal; the instrument used in the investigations on the patients in this thesis was the Doptek Spectrascan supplied by Doptek Ltd., Terminus Road, Chichester, West Sussex, UK. The frequencies carried in the shift signal are directly proportional to the velocities of the particles (cellular elements of the blood) in the sample volume. A frequency/time plot will therefore show the velocity of blood flow throughout the cardiac cycle. A normal trace from the internal carotid is shown in figure 5.9. Note the narrow band of frequencies obtained; this indicates that all the particles in the sample volume are moving at approximately the same velocity. This is a feature of laminar flow in which adjacent layers of fluid move parallel to one another and at velocities which differ

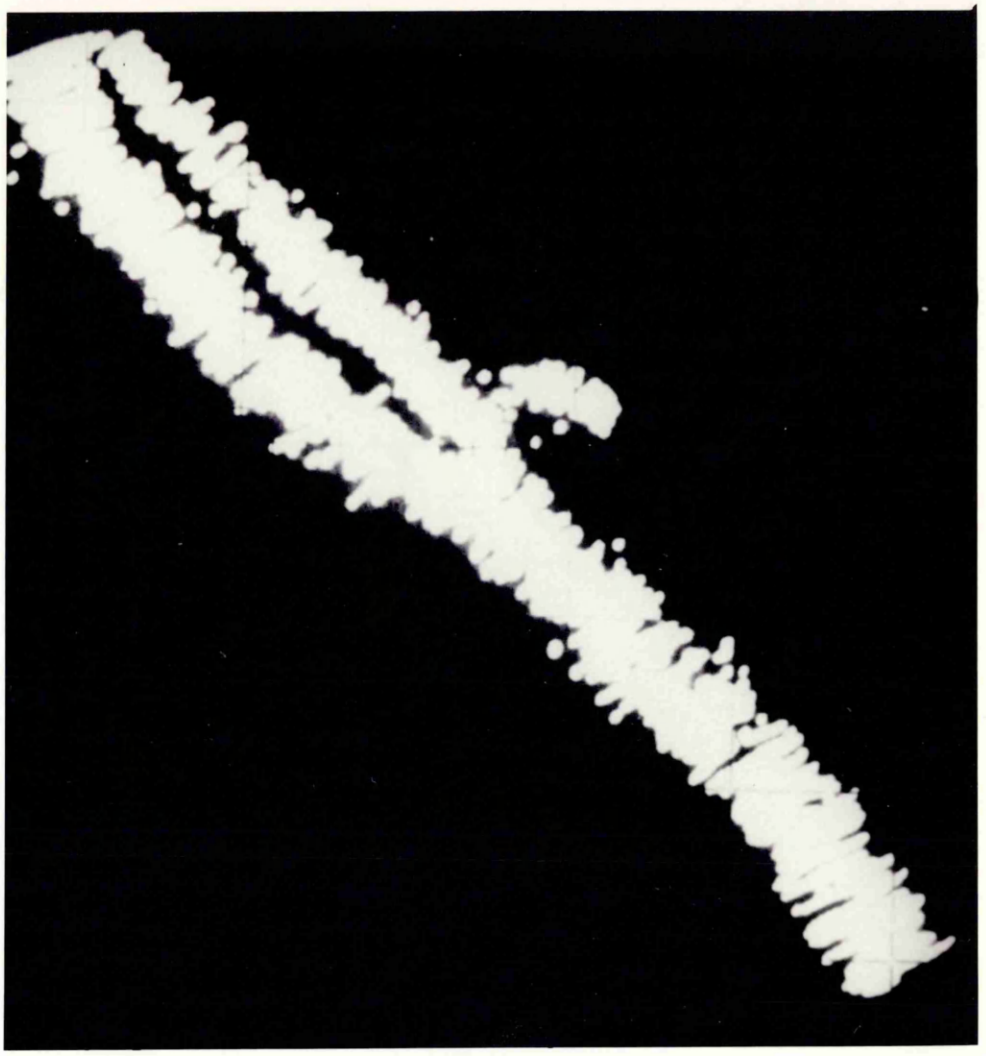

FIGURE 5.6

DOPPLER IMAGING: APPEARANCE OF NORMAL CAROTID BIFURCATION.

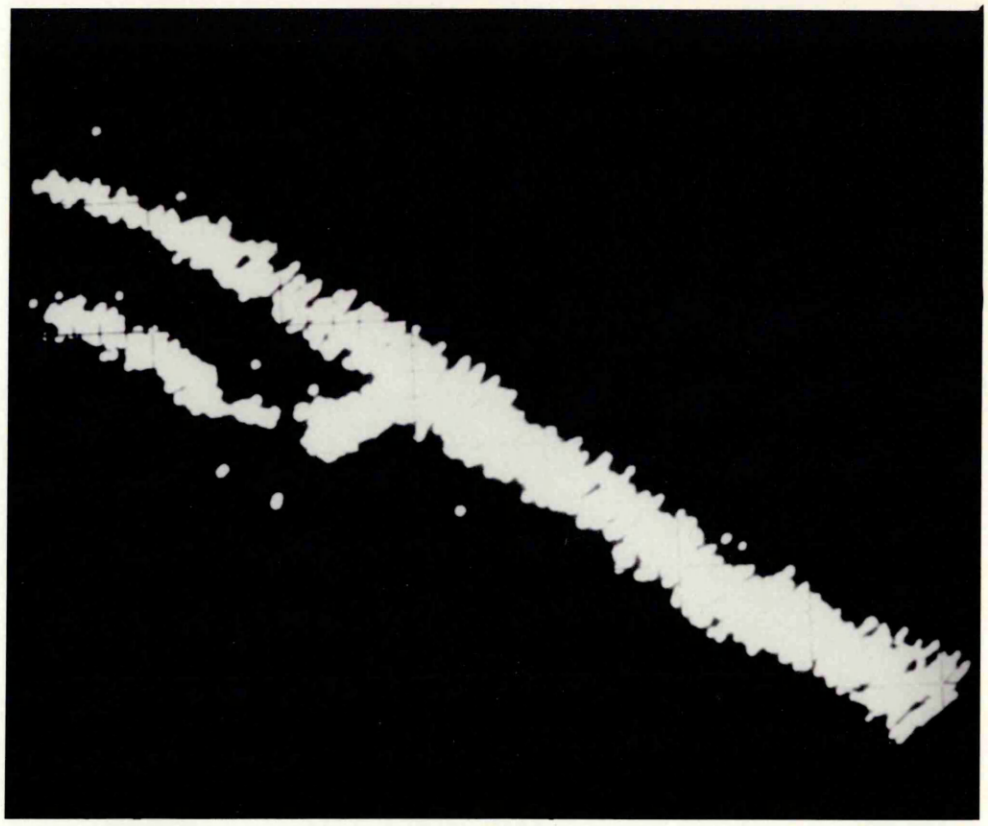

FIGURE 5.7

DOPPLER CAROTID IMAGE: STENOSIS OF INTERNAL CAROTID JUST DISTAL TO ITS ORIGIN.

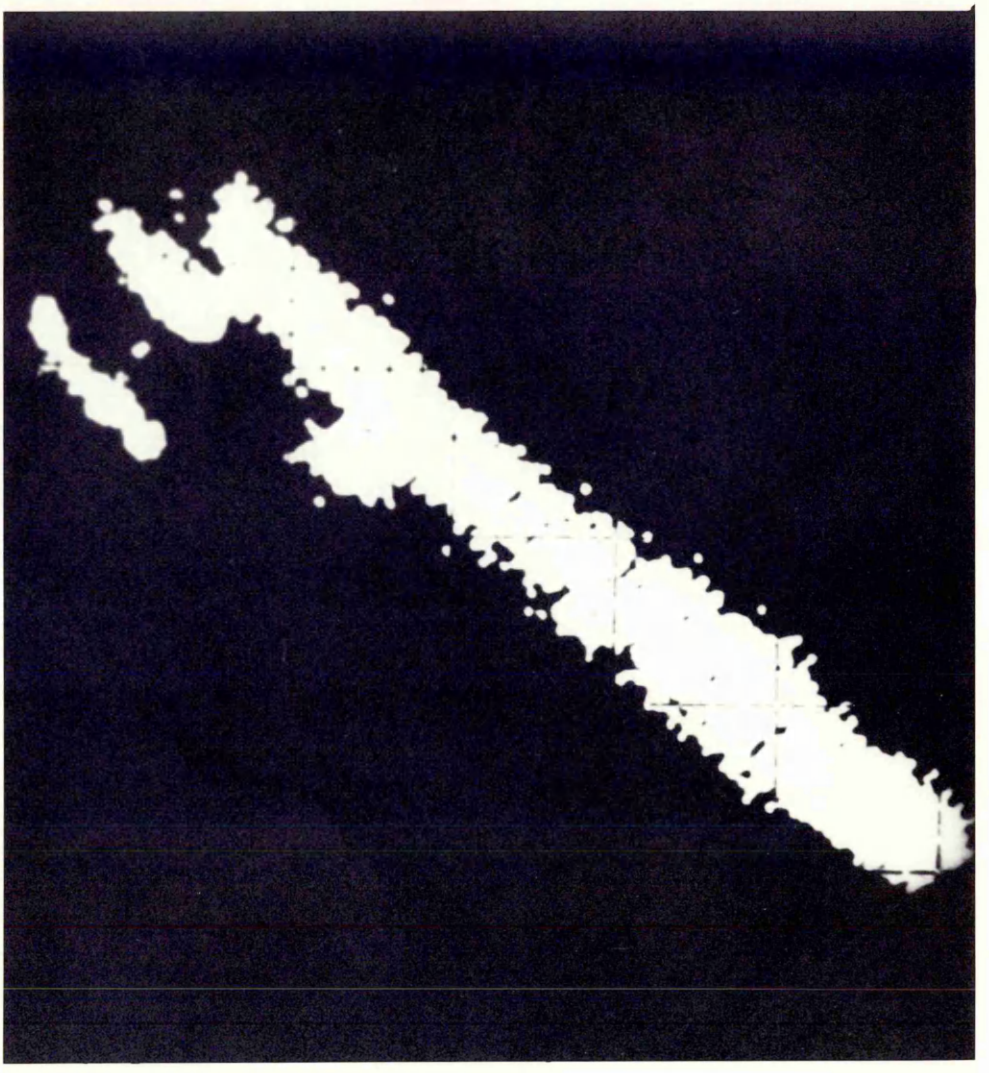

FIGURE 5.8

DOPPLER CAROTID IMAGE: INTERNAL CAROTID OCCLUSION.

The stump of the internal carotid is clearly visible and the vertebral artery is seen as a separate image on the extreme left.

only slightly. Examination of figures 5.10 and 5.11 which show the spectra from signals obtained at and just distal to a carotid stenosis, demonstrates two clear differences from the normal signal in figure 5.9. Firstly the peak frequency in systole is markedly increased and secondly the narrow band of frequencies previously present has been replaced by a much broader range - so-called spectrum broadening.

These two changes are a result of the changes which occur in flow downstream from a stenosis. Since volume flow remains unchanged, a reduction in luminal cross-sectional area produces an increase in mean velocity and this is reflected in the increased peak frequency detected by spectral analysis. This change is most obvious at or close to the stenosis. The second effect of diminished radius on flow characteristics is to produce a change from laminar to turbulent flow. Instead of fluid layers moving parallel, with the greatest velocities in the centre of the flow, a disorganised pattern of flow exists. A wide range of velocities is present among the cellular elements in the bloodstream and this results in a wide range of frequencies in the Doppler shift signal.

Broadening of the frequency spectrum may be quantified by physical measurement of permanent copies of the frequency/time spectrum. These may be obtained by a variety of methods including recording on heat sensitive paper or photographing screen images. In practice however, it has been found that an experienced operator can identify significant stenoses with equivalent accuracy by listening to the Doppler shift signal on earphones.

All of the TIA patients studied in this thesis had pulsed Doppler imaging and spectrum analysis of all three carotid vessels on both sides carried out. This examination normally takes under 30 minutes to

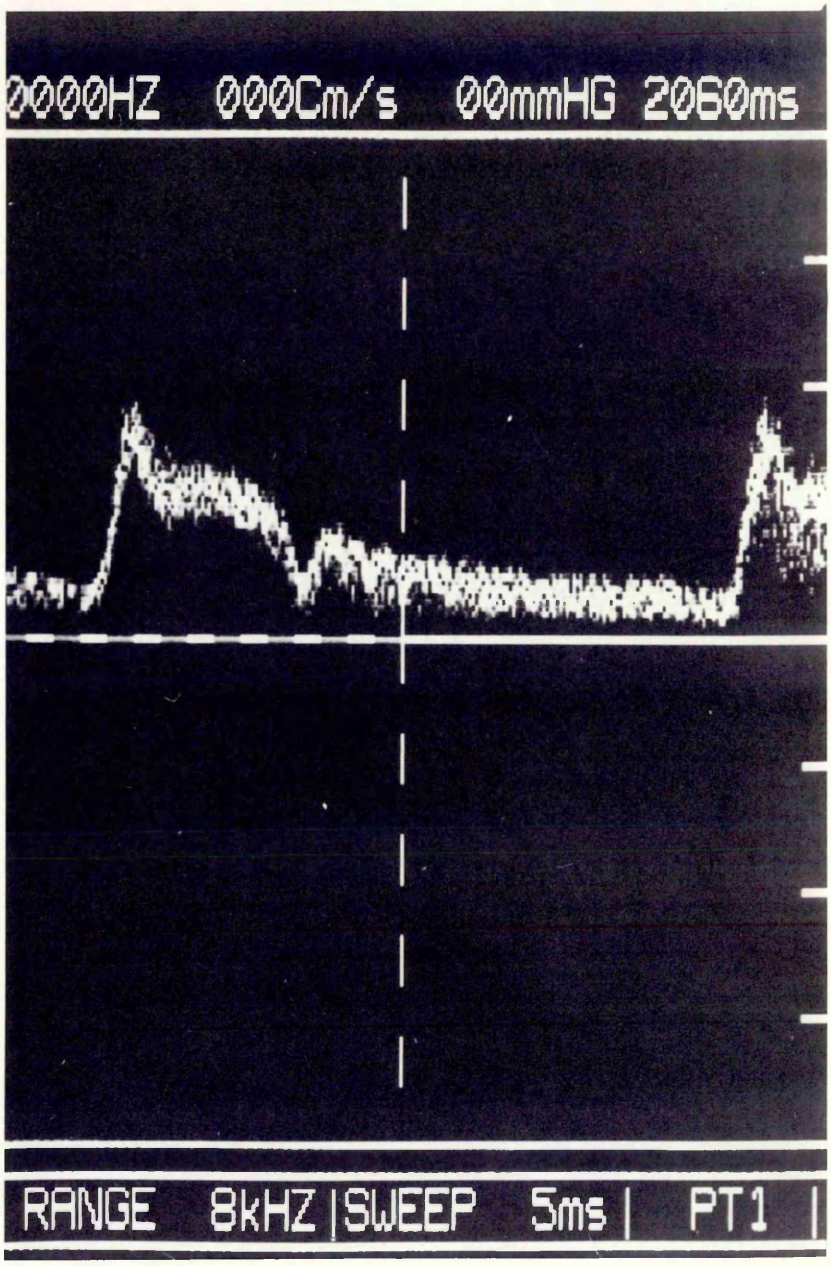

FIGURE 5.9

FREQUENCY/TIME SPECTRUM: NORMAL INTERNAL CAROTID TRACE.

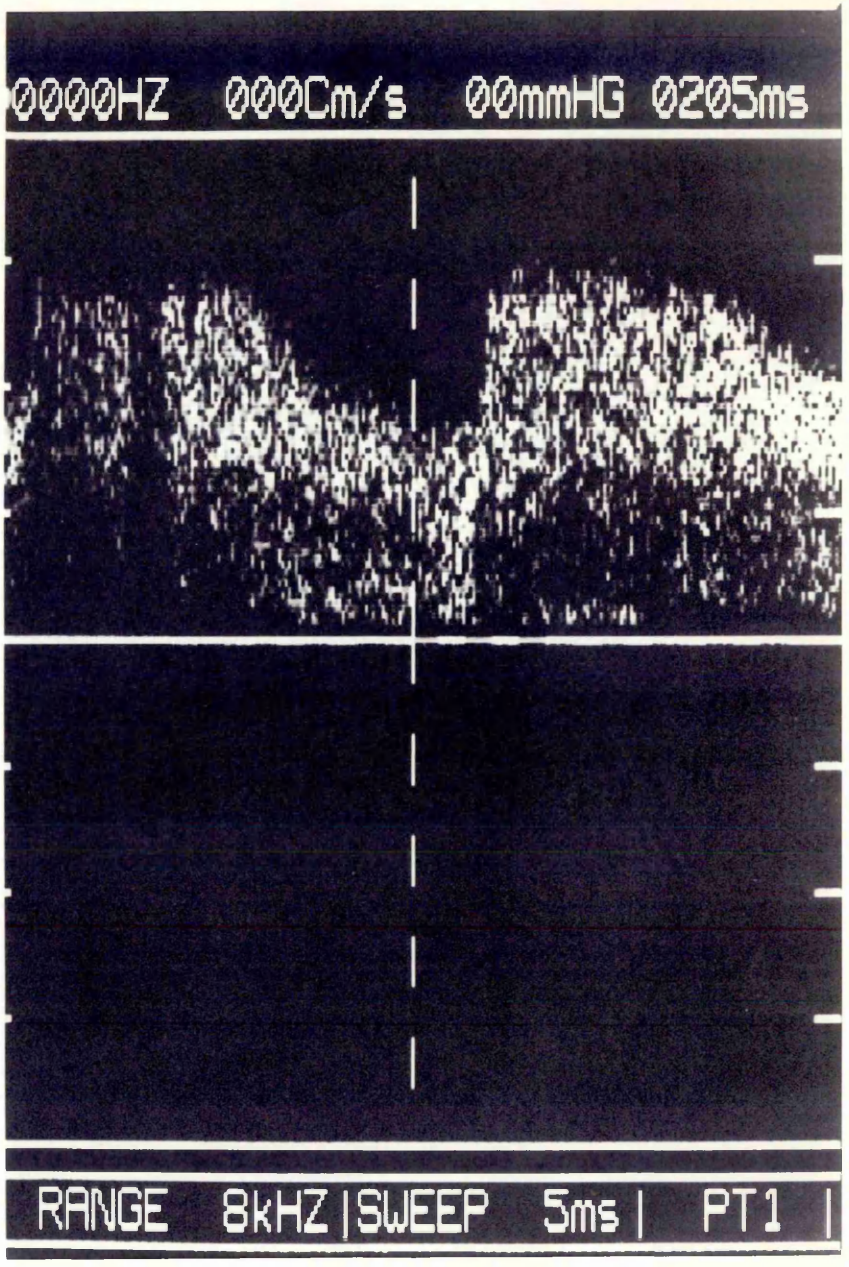

FIGURE 5.10

FREQUENCY/TIME SPECTRUM: TRACE FROM INTERNAL CAROTID STENOSIS.

Note (i) increased peak frequency and (ii) spectrum broadening

(compare figure 5.9).

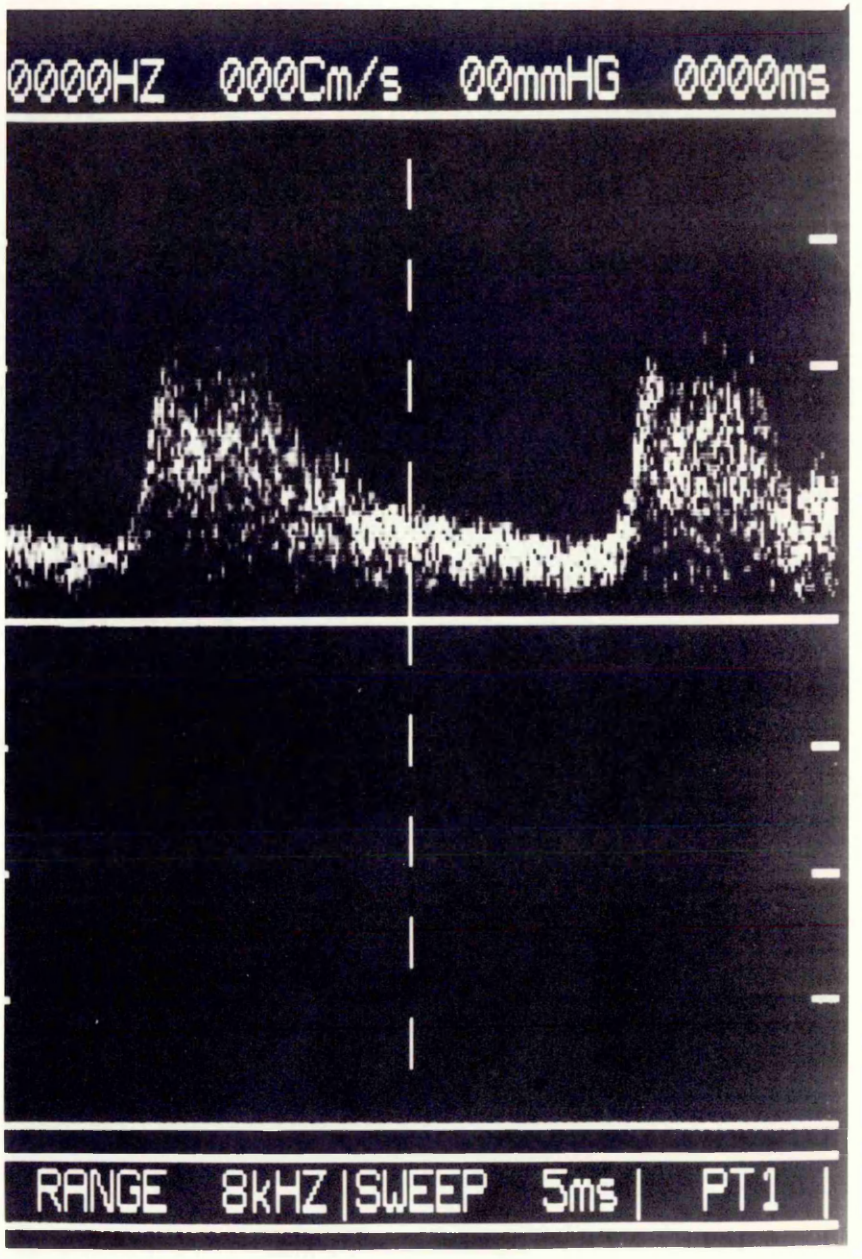

FIGURE 5.11

FREQUENCY/TIME SPECTRUM: TRACE FROM INTERNAL CAROTID DISTAL TO STENOSIS.

Note spectrum broadening (compare figure 5.9).

complete and is well tolerated by the patients. Routine procedure is to identify each of the vessels adjacent to the common carotid bifurcation and produce an image on the oscilloscope screen. An audio recording of the Doppler shift signal is made from each of the vessels and in addition frequency/time spectra are displayed and photographed for each vessel. Interpretation of each of these recordings is made by the investigator. Although this appproach has a subjective element, previous studies have shown that it is no less accurate than objective measurements of permanent records, provided the observer is adequately experienced. All of the studies carried out on patients in this thesis were performad by the same operator, who has several years of experience using these techniques. Reference to Doppler examination or Doppler scanning elsewhere in this thesis refers to the technique of combined Doppler imaging and Doppler spectrum analysis described in this section.

5.7 PATIENTS

All patients studied in this thesis were referred to the vascular laboratory in Gartnavel General Hospital, Glasgow for Doppler ultrasound examination of the carotid arteries. Sources of referral are varied but are mainly the Institute of Neurological Sciences at the Southern General Hospital and medical units in Gartnavel and associated general hospitals. There is also a significant number of patients referred by ophthalmologists with a provisional diagnosis of amaurosis fugax. Patients in this study are drawn entirely from the population of the West of Scotland.

The diagnosis of transient ischaemic attack was made initially in all cases by a neurologist, physician, or vascular surgeon and confirmed by the author. All cases of amaurosis fugax were seen initially by an ophthalmologist. At the time of interviews details of the patient's symptoms were noted along with information on symptoms and signs of other vascular disease. Relevant previous history was also noted with particular reference to myocardial infarction and hypertension. A detailed note of the patient's smoking habits was obtained.

The interval between the transient ischaemic event and referral to the vascular laboratory varied widely from a few days to several months. This variation was not thought to be important because viscosity parameters in an individual are not subject to significant change in the absence of marked physiological or pathological insult.

5.8 STATISTICAL ANALYSIS

In all of the studies described in this thesis clinical and other descriptive data were classified numerically and logged, together with all numerical data obtained from measurements, on the mainframe computer at Glasgow University. The categories of classification of non-numerical data are described, where relevant, in the various experimental chapters. The mainframe computer is an ICL 2988 manufactured by International Computers Ltd., and is run using the Virtual Machine Environment (VME) Operating System. Data were logged and edited using the Edinburgh Compatible Context Editor (ECCE) implemented under the VME system.

Statistical analysis of all data was carried out on the GVME computer using the MINITAB statistical package (Minitab Inc., 3081 Enterprise Drive, State College, Pa. 16801, USA.). MINITAB is a general purpose data analysis system designed for researchers who have little previous experience with computers and is consequently relatively simple to use. Despite its ease of use, it is flexible and fairly powerful, and readily capable of handling data sets larger than the one generated by the work in this thesis. It consists of a worksheet made up of rows and columns in which the data are stored. Manipulation, editing and analysis of the data are performed with a range of about 180 commands, whose functions are outlined in the Minitab Reference Manual (Minitab Inc.) and the Minitab Handbook (153).

To facilitate selection of appropriate statistical tests and check data logging, the distribution of all data sets and subsets was checked using a variety of facilities in the Minitab programme. Visual displays of data in the form of dotplots and histograms confirmed that all data sets conformed to a normal distribution. Comparisons between groups were made using a pooled t-test and values expressed as means ± the standard error. The null hypothesis was rejected when p was less than 0.05.

Chapter 6.

FOLLOW-UP OF DOPPLER-NORMAL TIA PATIENTS

6.1 INTRODUCTION

A large percentage of patients who present with TIA are subsequently found to have atheroma of the internal carotid, usually situated close to, or involving, the common carotid bifurcation (34,50,82-5). Of the remainder, a proportion will have cardiac disease which may provide a source of emboli or give rise to arrythmias which may in unusual circumstances produce focal cerebral deficits (Chapter 2). Haematological disease and other conditions will account for symptoms in a further few patients. However, exhaustive investigation of any group of TIA patients will identify a number whose symptoms are unexplained. The size of this group varies among different series but may be as large as 25% (34).

There are a number of possible explanations for this diagnostic difficulty. Firstly the diagnosis of the TIA may be incorrect. As previously observed (Chapter 2) a number of other conditions may closely mimic TIA and even experienced clinicians may be misled. In such cases the true diagnosis may become apparent in due course, although not revealed by appropriate investigation at the time of presentation.

Another possibility is that embolic sources are overlooked and this may occur for three reasons. They may be small, abnormally situated or the investigation used to detect them may be insufficiently sensitive. Ulcerated non-stenotic atheromatous plaques in the carotid may produce emboli (47) and yet remain undetected by arteriography. Similarly, atheromatous lesions in the more proximal vasculature, the origin of the common carotid artery and the aortic arch for example may escape detection, as indeed may more distal intracranial lesions. The failure to detect unusually sited lesions is of little practical clinical relevance because surgical correction is not normally feasible, but it may account for the 25% of patients whose TIAs are unexplained.

The sensitivity of investigations used to screen for carotid disease in particular has been extensively studied in recent years because of the increasing variety of non-invasive tests and the increase in their use. Sensitivity is a function both of the inherent accuracy of the test itself and the skill and experience of the investigating technician. Widely varying accuracies for non-invasive assessment of carotid artery disease have been reported (Chapter 2.4) and this partly explains the varying proportion of patients with TIA who are ultimately shown to have an embolic source. The operator dependent aspect of some investigations applies also to the use of ultrasound in the detection of cardiac valvular disease, another potential embolic source.

One further possibility worthy of consideration to account for unexplained TIAs is that their mechanism may be different from all other TIAs. It has been proposed for example, that abnormal platelet

function may cause TIAs in patients who lack other detectable pathophysiological mechanisms (154) and later in this thesis the role of abnormal blood viscosity in these patients will be discussed.

Whatever the reason for the symptoms in these patients it is of some interest to know their prognosis. If they have the same risk of subsequent stroke as untreated TIAs then close follow up and repeated or more extensive investigation of their symptoms may be warranted. On the other hand, if their symptoms constitute a single event with no important sequelae then no further care is required. The outcome in this group of patients may vary from centre to centre depending on the screening techniques used and the skill with which these are applied as noted above. There may also be national and regional variations in the incidence and patterns of arterial disease and it is therefore necessary to establish the clinical outcome of local patients with TIAs. For this reason it was thought necessary to follow up a group of these patients who had non-invasive carotid evaluation carried out in the vascular laboratory in Gartnavel General Hospital.

6.2 PATIENTS & METHODS

The vascular laboratory in Gartnavel General Hospital, Glasgow receives referrals for assessment of carotid disease from a wide variety of sources: neurologists, neurosurgeons, vascular surgeons, ophthalmologists and general physicians, both from within the hospital and from other institutions. Because Doppler examination of the carotids is free from complications and because access to the service is largely unrestricted the reasons for referral are many and varied. In order to concentrate on patients with a firm diagnosis of carotid

territory TIA it was decided to review two groups of patients. The first group comprised those who had been referred from the Institute of Neurological Science (INS) at the Southern General Hospital for assessment of hemispheric symptoms. In practice all of these patients are referred by, or on the instructions of, a senior neurologist or neurosurgeon. The second group of patients were selected from those identified in a previous study of amaurosis fugax (155).

Group One

In the period 1981-1985 all patients referred from the INS with hemispheric symptoms and who had normal Doppler examinations were considered for review. Initial information on these patients was obtained by examination of their records and follow-up was carried out by postal questionnaire, telephone interview, or in some cases by contact with the patient's general medical practitioner. Particular attention was paid to establishing the initial diagnosis and the occurrence of relevant subsequent events. In the follow-up review patients were asked specifically about recurrence of initial symptoms, new neurological symptoms, and any periods of in-patient hospital care.

Group Two

A separate review of patients with amaurosis fugax was carried out in a similar manner. These patients were initially identified in a previous study (155) and follow up data was obtained using the same techniques of questionnaire administration and telephone interview. The initial study had been designed to discover the prevalence of haemodynamically significant carotid disease in a population of patients with amaurosis fugax. Two hundred patients were initially considered; of these one hundred and five were excluded from analysis for one or more of the following reasons: symptoms suggestive of

vertebrobasilar ischaemia, concurrent eye disease, migraine, permanent visual deficit at presentation, possible non-carotid embolic source or vague symptoms. By using these criteria it was intended that only patients with definite amaurosis fugax and without a possible cardiac embolic source would be considered. Patients with a positive Doppler examination were excluded and the remainder considered for follow-up in the present study.

6.3 RESULTS

Table 6.1. shows data on age, sex distribution and length of follow-up for both groups. It is noteworthy that the mean age in both groups (51 years and 52 years respectively) is low when it is considered that the peak incidence of stroke occurs in the seventh and eighth decades of life. The preponderance of males in both groups is also noticeable; this corresponds to the biased sex distribution which is apparent in young stroke patients. All patients in group two were followed for at least two years and although some patients in group one had a relatively short follow-up interval, the mean time to review in this group was 31.7 months, and 75% of these patients were followed for at least two years.

Group One

One hundred and seventy nine patients who had been referred with unilateral symptoms, and who had no carotid disease detected by pulsed Doppler examination, were identified. Insufficient data were available on 17 patients (9.5%) and they were excluded from the study. Of the remaining 162, a further 88 patients were excluded as follows:

-19 with symptoms suggestive of vertebrobasilar ischaemia.

	Group 1 n = 74	Group 2 n = 28
Mean age (range)	51 (29-70)	52 (22-78)
Sex (M : F)	51 : 23	17 : 11
Mean follow up (range) (months)	31.7 (6-57)	39.3 (24-65)

TABLE 6.1

PATIENT DATA : FOLLOW UP STUDIES

Symptom free	36	(48.6%)
Recurrent TIA (including AF)	27	(36.5%)
Stroke	6	(8.1%)
Death	5	(6.8%)
Total	74	

TABLE 6.2

OUTCOME OF TIA PATIENTS WITH NORMAL DOPPLER

Symptom free	9	(32.1%)
Recurrent AF (or TIA)	12	(42.9%)
Stroke	3	(10.7%)
Death	4	(14.3%)
Total	28	

TABLE 6.3

OUTCOME OF AMAUROSIS FUGAX PATIENTS WITH NORMAL DOPPLER

-18 with amaurosis fugax alone.

-43 with permanent or prolonged deficits at presentation (stroke or PRIND).

-8 patients in whom prolonged follow-up revealed non-vascular causes for their symptoms.

The results of follow-up of the residual 74 patients are summarised in table 6.2. Less than half (48.6%) were symptom-free at the time of review while almost an equivalent proportion (44.6%) had experienced recurrent transient ischaemic symptoms or a completed stroke. Of the five deaths in this group three were due to cardiovascular causes, one was due to carcinoma of the bronchus and the cause of the fifth is unknown.

Group Two

Twenty eight of the previously identified Doppler-normal patients were available for follow-up. The results in this group are detailed in table 6.3. and a high incidence of recurrent symptoms is again evident. Fewer than one-third of these patients were symptom-free at the time of review while more than half (53.6%) had suffered either recurrent eye symptoms, hemisphere TIAs, or a completed stroke. Although the mortality rate was high (14.3%), only one of these deaths was due to a cardiovascular cause.

6.4 DISCUSSION

There have been several studies which have examined the fate of patients with TIAs of unknown aetiology. In 1971 Marshall reported 64 patients who had had carotid distribution TIAs (excluding amaurosis fugax) and who had had normal angiograms (156). There were 11 deaths

during follow-up, five due to cardiovascular causes and two attributable to cerebrovascular disease. During the follow up period (5.4 years) 19 (36%) of the survivors had further manifestations of cerebrovascular disease in the form of TIAs or completed stroke. In addition, almost 20% developed symptomatic vascular disease in other sites. It was concluded from this study that patients with TIA in whom no aetiological cause could be discovered were at considerably increased risk from the subsequent development of clinically evident vascular disease. The cause of the TIAs remained obscure but a cardiac source of emboli was suggested by a number of features. Firstly there was a high incidence of ECG abnormalities among patients who developed complications. Secondly, myocardial infarction occurred in 15% of patients during follow up, and finally, there was no correlation between the side of the TIA and any subsequent stroke, which might have been expected if sub-clinical carotid disease was responsible.

In 1976 Toole (157) reported a series of 226 patients with TIA (both carotid and vertebrobasilar) investigated by angiography. No embolic source was identified in 16 patients who were then followed for a mean of 5.7 years. The incidence of subsequent vascular disease was high. Four patients developed strokes, two continued to have TIAs and one suffered a myocardial infarction. A total of five patients died. Toole noticed, as Marshall had done, that patients with associated disorders such as hypertension and myocardial disease at the time of presentation were at most risk of dying soon after.

A third follow-up study of TIAs with normal arteriograms has been reported in two stages by Evans and colleagues (158,159). Their most recent figures show that in 56 patients followed for a mean of 25 months only three have had further TIAs and one has had a myocardial

infarction. There have been no strokes. This suggests a more benign prognosis than either Marshall or Toole, but the follow up period is still short by comparison.

Of the studies mentioned so far each has used arteriography to investigate all patients with TIAs, in contrast to the current practice in Glasgow which is to reserve arteriography for those patients whose non-invasive evaluation suggests haemodynamically significant disease (>25% stenosis). However, one study which has followed patients investigated by non-invasive tests only, has been reported by Hershey (160). In this series, non-invasive assessment was carried out using a combination of oculoplethysmography (OPG) and carotid phonoangiography (CPA) which together have an accuracy approaching 90% when compared with angiography (160). Patients with a stenosis ≥50% and those with frequent "crescendo" TIAs were referred for arteriography and possible surgery. The remaining patients formed the group for review. Three hundred and fifty three patients were followed for a mean of about four years. In this group 7.7% developed strokes, 14.3% suffered recurrent TIAs and 17.5% had a myocardial infarction. It was observed that 14 of the 20 strokes in this series occurred after recurrent TIAs and that roughly one third of patients experiencing recurrent TIAs went on to have a completed stroke.

With the exception of the series reported by Evans, which has a shorter follow-up than the others, these studies indicate that TIAs for which no cause can be found do not have a good prognosis. The findings from the two surveys carried out for this thesis support this contention. The 8% stroke rate in group one and the 10% rate in group two represent crude annual rates of 3% and 5% respectively, not substantially different from the 5% annual incidence of stroke

following untreated TIA (21,70)(Chapter 1). Morbidity and mortality from vascular diseases other than cerebrovascular disease were also prominent features of all of the other studies, again with the exception of Evans's. Four of the nine deaths which occurred in our studies were due to cardiovascular causes.

The pathophysiological mechanism involved in the production of TIAs in these patients is not clear, but the high incidence of subsequent vascular disease suggests that it is related, at least in some cases, to arterial disease. The simplest explanation is that the investigations used to detect atheroma are not sufficiently sensitive. This may clearly be the case when non-invasive assessment is utilised. Non-stenotic or minimally stenotic carotid disease will not be detected by the pulsed Doppler imaging and spectral analysis used in Gartnavel. Although atheroma which produces stenoses of <25% does not produce sufficient flow disturbance to permit its detection by these methods, it may ulcerate, become thrombogenic and act as a source of emboli. The OPG/CPA routine used by Hershey disregarded stenoses of less than 50% and this may account for the relatively high stroke rate in his group (160).

Although conventional arteriography is the standard by which non-invasive tests are judged, it is by no means infallible and it is possible that minimal arterial disease may escape detection, particularly if insufficient views are taken or if the proximal and distal vasculature are not examined. Progression of minimal arterial disease, overlooked at initial examinations, may thus be responsible for recurrent symptoms or the development of stroke. Since this disease is likely to be in the early stages of development when the patient first presents, it is possible that the interval to the

development of stroke may be longer than usual and this in turn may account for the lower incidence of complications in the single study with a short follow-up (159).

6.5 SUMMARY

The prognosis of patients who present with carotid distribution TIAs, including amaurosis fugax, and who do not appear to have atheromatous disease of the carotid bifurcation, may not be benign. The studies presented here suggest that there is a high incidence of recurrent symptoms and an increased risk of stroke and other cardiovascular disease in these patients when compared to the normal population. Most of the evidence in the available literature supports this view. This finding is at variance with what had been assumed as the relevance of a "normal" Doppler carotid scan, and has meant that follow-up of such patients has had to be changed. Further study of this group of individuals, particularly with regard to the aetiology of their symptoms, seems warranted.

Chapter 7.

RHEOLOGICAL COMPARISON OF TIA PATIENTS WITH AND WITHOUT SIGNIFICANT CAROTID DISEASE

7.1 INTRODUCTION

In chapter 4.2. it was observed that although Poiseuille's law imprecisely described flow in the conductance vessels of the circulation, it was useful in identifying the major factors influencing flow: the driving pressure, the vessel radius and the viscosity of the blood. TIAs are due to local failure of perfusion and it is apparent that this may occur for three reasons: inadequate driving pressure, diminished vessel radius or increased blood viscosity. Each of these three possible changes has been established as an aetiological factor in the causation of TIA. Reduced driving pressure (systemic hypotension) may produce perfusion failure although focal signs will only result with the additional presence of a stenosis which will selectively reduce flow (Chapter 2.2.) Increased blood viscosity has loosely been implicated in the pathogenesis of TIA by the relatively high incidence of focal ischaemia in patients with polycythaemia. As discussed previously (Chapter 2.2.) the way in which increased haematocrit leads to TIA is complex. Decreased cerebral blood flow (CBF) is produced by autoregulation in response to increased arterial oxygen content and possibly increased viscosity. In certain circumstances - hypotension, coincident cerebral atheroma -

this reduced CBF may lead to stasis and thrombogenesis. Focal ischaemia is opposed by local autoregulatory mechanisms but these have physiological limits which may be exceeded in the presence of disease. Under these circumstances perfusion is passive, ie, no longer determined by changing vessel radius, and increased viscosity may be relevant.

There is evidence to suggest that at the margins of areas of cerebral infarction, maximal vasodilatation occurs and perfusion is dependent on perfusion pressure and blood viscosity (130). Harrison et al discovered that in patients with occluded internal carotid arteries and completed stroke, the size of the cerebral infarct measured by computed tomography was related to the level of the haematocrit (129). They speculated that the increased viscosity and lowered cerebral blood flow associated with elevated packed cell volumes adversely affected the development of collateral circulation. In this situation a decrease in blood viscosity might be expected to improve circulation in these critical marginal regions and limit the extent of infarction. This is partly the basis for haemodilution therapy in stroke (Chapter 4.7).

It seems that blood viscosity may be a relevant factor in local and global cerebral perfusion in some circumstances. The possibility that abnormal blood viscosity may contribute to the symptomatology of patients with TIAs, which are otherwise unexplained, was considered. In his initial report on the influence of blood rheology changes in patients with intermittent claudication, Dormandy observed that there was a subgroup of patients in whom the primary or main abnormality was increased viscosity (114). This chapter describes a study designed to explore the possible rôle of increased blood viscosity in the genesis of otherwise unexplained TIAs. Patients without non-carotid causes for

their symptoms were studied (ie, any patient with a cardiac or haematological cause for their symptoms was excluded). They were divided into two groups on the basis of the presence or absence of carotid disease and blood viscosity profiles were measured in each group. The results were compared to look for a viscosity effect in the patients without carotid disease.

7.2 METHODS

Patients with a history of TIA (including amaurosis fugax) were selected from those attending the vascular investigation laboratory in Gartnavel General Hospital. On the basis of history and examination, routine full blood count, serum biochemistry, and twelve lead ECG, those who had possible cardiac or other non-carotid causes for their symptoms were excluded. The following data were recorded for each patient studied: sex, age, pattern of TIA, history or physical findings of arterial disease, history of hypertension or anti-hypertensive medication, sitting blood pressure and smoking habits. In addition the results from routine haematological and biochemical tests were recorded. All patients had Doppler carotid imaging and spectrum analysis on both sides carried out by one operator. A Doppler scan result was considered positive if carotid occlusion or stenosis of at least 25% was detected ipsilateral to the symptomatic hemisphere (Chapter 5.6). (In no patient in this series was isolated contralateral disease discovered.) Blood for viscosity measurements was routinely withdrawn at the out-patient clinic visit following the Doppler studies. The timing and method of sample analysis was as described in Chapter 5.5. Whole blood viscosity was

measured at high ($94.5s^{-1}$) and low ($0.945s^{-1}$) shear rates and expressed both as native values and corrected to a haematocrit of 45%. These shear rates were selected as convenient machine settings which were as close as possible to the rates recommended by the International Committee for Standardisation in Haematology (144). Haematocrit for the correction was estimated by the microhaematocrit method (Chapter 5.3). Plasma viscosity and plasma fibrinogen levels were also determined by methods previously described (Chapter five)

Statistical analysis was carried out using MINITAB (Minitab Inc.) on the Glasgow University mainframe computer (VME).

7.3 RESULTS

A total of fifty patients were studied and the relevant characteristics of both groups are summarised in table 7.1. Twenty patients (40%) had carotid artery disease detected by Doppler investigation, and since this was a consecutive series it is assumed that this is representative of the population referred for evaluation in Gartnavel. It compares with incidences of between 31% and 78% as the proportion of all TIA patients shown to have internal carotid disease (34,50,82-5)(see Chapter 2.4).

Blood viscosity may be influenced by age (137,161), sex (161,162), smoking (137,163) and hypertension (136) and the distribution of these variables between the groups is therefore of some importance. Table 7.1 shows the mean age of the Doppler-normal group (54.3 years) to be markedly less than the Doppler-abnormal group (65.3 years), but very similar to the mean ages of the patients in the two follow-up groups described in the last chapter (51 and 52 years).

	Doppler Abnormal	Doppler Normal
Number of patients	20	30
Male : Female	12 : 8	19 : 11
Age (mean) yrs.	65.3	54.3
Smoking habit % (Never:Current:Former)	5:65:30	23:54:23
Hypertensives	9	9

TABLE 7.1

TIA PATIENT DATA

The sex distribution between groups is similar with a preponderance of men in both groups. While this might have been expected in the younger Doppler-normal group, the mean age of the other group is sufficiently high for the benefit of female sex, in respect of reduced risk of arterial disease, to be no longer apparent. This anomaly is presumably the result of the relatively small numbers under study.

The effect of smoking on viscosity has been well documented. This effect is partly reversible, and for this reason non-smokers were recorded either as former smokers or never having smoked (143). The distribution of patients among the three smoking categories shows a slight preponderance of smokers in the Doppler-abnormal group (X^2=3.008, NS).

Patients were decreed to be hypertensive if they were receiving anti-hypertensive medication or if their diastolic blood pressure exceeded 90mmHg (164).There is a greater proportion of hypertensives in the Doppler-abnormal group (X^2=1.172, NS).

Native whole blood viscosity measurements at high and low shear rates are shown in table 7.2 along with values corrected to a haematocrit of 45%, and haematocrits. No statistically significant differences in any of these measurements were observed between groups, although examination of 95% confidence intervals indicated that small differences could not be excluded by this study.

Table 7.3 shows plasma viscosity values with relative high and low shear viscosities and plasma fibrinogen values. Relative high shear viscosity, which is the ratio of high shear viscosity, corrected to a haematocrit of 45%, to plasma viscosity, may be used as a measure of red cell deformability (RCD)(144). Relative low shear viscosity, the ratio of corrected low shear viscosity to plasma viscosity, may similarly be used as a measure of red cell aggregation (RCA).

	Doppler Abnormal	Doppler Normal
WVB - mPa.s		
High shear : native	5.01 ± 0.17	4.93 ± 0.14
: corrected	5.30 ± 0.15	5.23 ± 0.09
Low shear : native	18.00 ± 0.86	17.68 ± 0.92
: corrected	19.79 ± 0.77	19.54 ± 0.60
Haematocrit	43.00 ± 0.82	43.07 ± 0.79

WBV = Whole blood viscosity

TABLE 7.2

TIA PATIENTS: WHOLE BLOOD VISCOSITY AND HAEMATOCRIT

	Doppler Abnormal	Doppler Normal
Plasma viscosity mPa.s	1.41 ± 0.02	1.38 ± 0.02
RCD	3.78 ± 0.11	3.77 ± 0.06
RCA	14.09 ± 0.52	14.05 ± 0.35
Fibrinogen g/l	4.20 ± 0.22*	3.60 ± 0.19*

* p = 0.041

RCD = Red cell deformation
RCA = Red cell aggregation

TABLE 7.3

TIA PATIENTS: PLASMA VISCOSITY, RELATIVE VISCOSITIES AND FIBRINOGEN

Fibrinogen levels in the Doppler-abnormal group are higher than in the Doppler-normal group and this difference is statistically significant ($p = 0.041$). This difference is reflected in the higher plasma viscosity in the Doppler positive group although the plasma viscosity difference does not reach statistical significance. Relative viscosities in both groups are similar.

7.4 DISCUSSION

Cerebral perfusion is normally maintained by autoregulation, which has been defined as the "intrinsic tendency of an organ to maintain constant blood flow despite changes in arterial perfusion pressure" (165). Several organs including kidney, liver, and skeletal muscle as well as brain, exhibit this property and different mechanisms have been postulated to account for it. In the cerebral circulation, autoregulation is probably accomplished by a combination of mechanisms which respond to changes in perfusion pressure, or local metabolic alterations. Vascular smooth muscle may respond directly to these changes or they may be mediated by changes in autonomic tone (130). Whatever the mechanisms involved, normal CBF can be maintained over a wide range of arterial pressures in a normal individual (166). The ability of autoregulation to preserve CBF may be compromised in disease however, and under these circumstances the range of arterial pressures over which blood flow can be maintained is narrowed. Beyond the limits of autoregulation blood flow becomes passive and is directly related to perfusion pressure; under these circumstances blood viscosity may be an important determinant of flow (130).

In the pathogenesis of TIA elevated blood viscosity could contribute in two ways. Firstly in patients in whom local autoregulation is impaired by pre-existing disease, if blood viscosity is sufficiently elevated the resultant increased yield stress may exceed the perfusion pressure and stasis will result. In this situation minor changes in systemic blood pressure may be sufficient to produce intermittent stasis and ischaemia. Support for this hypothesis is provided by Hutchison's study which demonstrated an increased yield stress in TIA patients (167). The second mechanism by which increased blood viscosity could predispose individuals to TIA is by inhibiting the development of collateral circulation when vascular occlusion does occur. Collateral circulations are by nature circuitous and often via narrow channels; since, according to Poiseuille's law, an increase in tube length and a decrease in radius both adversely affect flow, the effect of any added viscosity changes in these circumstances will be greatly magnified.

The results of the study presented here show little difference between whole blood viscosity in patients with and without detectable carotid disease. It seems unlikely therefore that symptoms in patients without detectable embolic sources are due to abnormally elevated viscosity. Indeed the small differences which have been demonstrated are all in the opposite direction; patients with carotid disease tended to have slightly higher blood viscosities although the differences are not statistically significant. These differences may be explained by the observed differences in fibrinogen levels, which are elevated in both groups. This elevation may well be related to vascular disease or it may simply be a non-specific reaction to

illness, since fibrinogen is an acute phase reactant. The cause of symptoms in patients without a demonstrated embolic source remains obscure but a number of possibilities can be suggested.

The most obvious suggestion with regard to the present study is that a minor degree of carotid disease may be present. The overall accuracy of Doppler ultrasound in the detection of carotid disease as used here is 96% for stenosis >25% (92). However non-stenotic or minimally stenotic disease does not produce the turbulent flow which is necessary for a positive test and remains undetected. While atheroma of this degree is less likely to form thrombus and carries a much lesser risk of subsequent stroke than disease which is significantly stenotic (46), there is little doubt that it may be a source of emboli particularly if it is ulcerated (47,48,168). Some patients may therefore have lesser degrees of carotid disease to account for their symptoms but this cannot explain them all. In previous studies of this group of patients angiography was used to assess the carotids and this would be expected to detect most minor irregularities; even so there was still a proportion of patients whose symptoms remained unexplained (156,157,159). Similarly, intracranial atheroma could be expected to account for the symptoms of a number of patients in this study but this too has been investigated in other studies and it does not account for all individuals (156,157). One area where angiography might be expected to miss significant atheroma is in the proximal vasculature; aortic arch lesions are difficult to demonstrate and may be presumed to be responsible for some embolic events. It is reasonable to suppose that minor, undetected arterial lesions, in a variety of vessels, are the site of thrombosis and subsequent embolism in a proportion of patients (169), but the fact

that even when a careful search with reliable methods is made for such lesions, 25% of patients have none found (34) suggests that other mechanisms are involved.

As discussed previously (Chapter 2.2.) the heart is not infrequently implicated as a possible source of embolic material and although most patients with relevant lesions will be identified by examination of the cardiovascular system and a standard 12 lead ECG, some conditions require more elaborate investigations to uncover appropriate pathology. Mitral valve prolapse for example, has recently been recognised as a more frequent cause of cerebral embolism than had initially been thought (58) and its identification depends on cardiac ultrasound. Although the overall contribution of this diagnosis to the pathophysiology of TIA is small, it is proportionately much more frequently encountered in symptomatic younger individuals. There is a case for investigating all patients under the age of 45 who have unexplained TIAs by cardiac sonography to look specifically for this condition. Unrecognised valvular disease probably contributes in some measure to the group with unexplained symptoms. The mean age of this group in the present study was only 54.3 years and contained a significant proportion of patients less than 45 years of age.

Abnormal platelet numbers or function have been suggested as causes of TIA. Primary thrombocytosis as a cause of cerebral symptoms, including TIA and amaurosis fugax, has been described by Bernad (170). In 26 of the 28 patients whom he reviewed, platelet counts were in excess of 1 million/mm^3; a reduction in platelet count following therapy prevented recurrent TIA. Patients in this category should be readily identifiable. Other platalet abnormalities may not be so easily recognised. Platelet adhesiveness has been shown to be increased in young patients with thromboembolic stroke (119) but the

relevance of this finding to the occurrence of the stroke is unclear. In a study comparing TIA patients with controls Olsson (171) was unable to demonstrate any overall differences in platelet function or in the numbers of circulating platelet aggregates (CPA). However individual patients had high numbers of CPA and enhanced platelet aggregation. Al-Mefty and his colleagues reported 22 patients with TIA in whom no cause for their symptoms could be found after appropriate investigation by angiography, isotope brain scan, cardiac ultrasound and ECG (154). All patients had abnormalities of platelet adhesiveness and/or aggregation demonstrated during their investigations. Treatment with aspirin and/or dipyridamole or sulphinpyrazone produced improvements in the abnormal platelet studies during follow-up and prevented recurrence of TIA; in two cases discontinuation of treatment was associated with recurrent symptoms and a deterioration in platelet function. It is perhaps noteworthy that of these 22 patients 15 were less than 50 years of age.

All of this evidence suggests that abnormal platelet function leading to the production of platelet emboli may underlie the diagnosis of TIA in some patients. This may be particularly relevant in young patients. However the possibility that abnormal levels of circulating platelet aggregates result from, rather than cause, cerebral thromboembolism in many patients must also be considered.

In summary it appears that some TIAs of unknown aetiology are due to embolic sources, either arterial or cardiac, undetected by screening procedures, or platelet abnormalities; an incorrect diagnosis of TIA which eventually becomes apparent will account for others (eg, eight patients in the follow-up study presented in chapter

6). It is possible that as yet undiscovered mechanisms will explain some TIAs but the number of patients involved in any such group is likely to be small.

Although the study in this chapter failed to reveal any rôle for elevated blood viscosity in the genesis of idiopathic TIAs it did suggest that fibrinogen levels are elevated in all TIA patients. In order to explore this further and to assess the effect that elevated fibrinogen may have on whole blood viscosity in these patients, a study comparing TIA patients with normal controls and with patients with established peripheral vascular disease, in whom viscosity abnormalities are well documented, was set up. This is reported in the following chapter.

7.5 SUMMARY

Screening patients with TIAs using Doppler ultrasound at Gartnavel General Hospital categorised carotids as either occluded, stenosed or normal. For patients with occluded or stenosed vessels a rational management plan had been evolved, but the management of Doppler-normal patients had not been fully rationalised. Aetiologies for these patients' symptoms had been considered and it was thought possible that viscosity abnormalities might be the mechanism of their symptoms. The possible rôle of abnormal blood viscosity in the genesis of TIAs of unknown aetiology was therefore investigated. No differences in whole blood viscosity, or haematocrit, between patients with carotid stenosis and those without, were demonstrated. However, plasma fibrinogen was elevated in both groups and was significantly higher in the group with demonstrable carotid disease. Thus, while

raised blood viscosity does not appear to explain the symptoms of patients whose TIAs are otherwise idiopathic, haemorheological abnormalities may be present in all TIA patients and may reflect changes due to arterial disease.

Chapter 8

HAEMORHEOLOGY OF TIA; COMPARISON WITH PERIPHERAL VASCULAR DISEASE AND NORMAL CONTROLS

8.1 INTRODUCTION

In chapter 4.1 evidence was presented which supports the hypothesis that blood viscosity is linked to cerebrovascular disease. In particular it was observed that blood viscosity is elevated in patients who have had a thromboembolic stroke and it remains elevated during convalescence from stroke. It is possible that these changes in blood rheology arise at least partly as a consequence of the ischaemic event but it is equally possible that they are antecedent to the event and play some part in its genesis. Such a situation exists in patients with ischaemic heart disease, in that rheological changes are apparent in patients with angina (110,111) but are more obvious following a myocardial infarction (108). The implications of such findings are clear. If rheological abnormalities contribute to the risk of subsequent ischaemic events then it may be possible to diminish that risk with therapy designed to correct abnormal viscosity. If patients with TIA are haemorheologically abnormal it would suggest that: a) such abnormalities may contribute to the stroke risk associated with TIA and b) rheological manipulation may be beneficial.

In order to assess the haemorheology of patients with TIA a group of these patients was studied, along with a matched group of control patients who had no clinical vascular disease, and a third group with intermittent claudication. This last group was included because viscosity changes associated with this condition have been well documented and are related to the presence of significant atheroma, which is also the underlying pathology in the majority of patients with TIA.

8.2 PATIENTS & METHODS

Group One. Patients with TIA (including amaurosis fugax) were selected from those attending the vascular laboratory for carotid scanning as described in chapter 7.2.

Group Two. Control subjects came from two main sources. One group comprised surgical inpatients, or outpatients, admitted for minor procedures such as herniorrhaphy or varicose vein surgery. No patient with clinically apparent vascular disease, undiagnosed symptoms, malignant disease, infection or recent surgery was included in this group. The second group of control subjects were members of the hospital staff. Again care was taken to exclude those with symptomatic vascular disease or other relevant illnesses. All control subjects provided details of their smoking habits and any history of hypertension, including therapy. Blood pressure was measured with a standard pneumatic cuff with the subject in a sitting position.

Group Three. Patients with intermittent claudication were selected from among those attending the peripheral vascular disease outpatient clinic, or were inpatients admitted for peripheral vascular

surgery. Again no patient with other significant disease or recent surgery was included. Patients with ischaemic rest pain, ulcers or gangrene were excluded. Age and sex, which have important influences on blood viscosity, were matched between groups.

The following measurements were carried out on each individual studied: whole blood viscosity at high ($94.5s^{-1}$) and low ($0.945s^{-1}$) shear rates, plasma viscosity, haematocrit, plasma fibrinogen. In addition, a full blood count was carried out and serum biochemistry was estimated. Derived values were calculated as follows: blood viscosity corrected to a haematocrit of 45% at both shear rates; relative blood viscosity at high and low shear rates (RCA, RCD). All measurements and calculations were performed exactly as described in chapter 5.2 - 5.5. Statistical analysis was carried out using methods and procedures described in chapter 5.8.

8.3 RESULTS

Fifty patients with a diagnosis of TIA were studied and compared with 20 claudicants and 64 control subjects. Comparative data on sex distribution, age, and smoking habits are shown in table 8.1. The sex distribution is similar in all groups. The mean age of the control subjects (54.3 years) is a little less than either of the patient groups (58.7 and 60.4 years). Smoking habits were categorised as previously outlined in chapter 7.3: non-smokers, current smokers and former smokers. Apart from a slightly larger proportion of individuals who had never smoked in the control group, smoking habits were comparable.

	TIA	Control	PVD
No.	50	64	20
M : F	31 : 19	39 : 25	12 : 8
Age (mean) yrs.	58.7	54.3	60.4
Smoking % (Never:Curent:Former)	16:60:24	23:63:14	15:70:15
Hypertensives	18	11	4

TABLE 8.1

CLINICAL DATA: PATIENTS AND CONTROL SUBJECTS

Whole blood viscosity (WBV) results are shown in table 8.2; both native viscosity and viscosity corrected to a haematocrit of 45% are shown. There are no significant differences between TIA patients and controls in any of the values shown. The patients with peripheral vascular disease (PVD) on the other hand were found to have significantly higher native viscosities, at both shear rates, than either of the other groups. These differences diminish, but do not disappear, when viscosity is corrected for haematocrit; haematocrit is higher in the PVD group although the difference does not reach statistical significance. At low shear rate, even when viscosity is corrected for haematocrit, PVD patients have a greater viscosity than the TIA group suggesting that either red cell properties (deformability or aggregability) or plasma viscosity differs significantly between these groups, in addition to haematocrit differences.

Table 8.3 shows plasma viscosity, relative viscosities and plasma fibrinogen levels for all three groups. Plasma viscosity is highest in the PVD patients but there is no significant difference between them and the TIA patients; the values for both of these groups are significantly higher than those for the control group. These differences are mirrored by the differences in plasma fibrinogen levels which must be at least partly responsible for the differing plasma viscosities. Relative high shear viscosity and relative low shear viscosity, measures of red cell deformation and aggregation respectively, show differences between the TIA and control groups indicating that the red cells from the former group deform more and aggregate less than those from the latter group.

	TIA	Control	PVD
WBV - high shear : mPa.s			
- native	4.96• ± 0.11	4.97* ± 0.10	5.36•* ± 0.12
- corrected	5.26 ± 0.07	5.28 ± 0.06	5.39 ± 0.09
WBV - low shear : mPa.s			
- native	17.81° ± 0.64	18.43* ± 0.69	21.08°* ± 0.81
- corrected	19.36* ± 0.47	20.30 ± 0.33	21.21* ± 0.54
Haematocrit	43.04 ± 0.58	43.11 ± 0.64	44.76 ± 0.64

WBV = Whole blood viscosity

* $p < 0.05$

• $p < 0.05$

° $p < 0.01$

TABLE 8.2

PATIENTS AND CONTROLS: WHOLE BLOOD VISCOSITY AND HAEMATOCRIT

	TIA	Control	PVD
Plasma viscosity mPa.s	1.397* ± 0.016	1.330*• ± 0.011	1.452• ± 0.026
RCD	3.781° ± 0.056	3.945°** ± 0.052	3.713** ± 0.093
RCA	14.06°° ± 0.29	15.15°° ± 0.24	14.60 ± 0.49
Fibrinogen g/l	3.83• ± 0.15	2.89•* ± 0.31	4.01* ± 0.26

RCD = Red cell deformation
RCA = Red cell aggregation

*	$p < 0.001$
•	$p < 0.001$
°	$p < 0.05$
**	$p < 0.05$
°°	$p < 0.01$

TABLE 8.3

PATIENTS AND CONTROLS:
PLASMA VISCOSITY,RELATIVE VISCOSITIES AND FIBRINOGEN

	T.I.A.	Control
Haemoglobin (g/dl)	14.35 ± 0.22	14.17 ± 0.22
Red cell count ($x10^{12}$)	4.56 ± 0.062	4.466 ± 0.070
MCV (fl)	91.90 ± 0.84	93.29 ± 0.80
MCH (pg)	31.48 ± 0.33	31.81 ± 0.31
MCHC (g/dl)	34.24 ± 0.13	34.08 ± 0.98

MCV = Mean corpuscular volume
MCH = Mean corpuscular haemoglobin
MCHC = Mean copuscular haemoglobin concentration

TABLE 8.4

TIA AND CONTROL PATIENTS: CELLULAR MORPHOLOGY

In the absence of direct measurements of red cell deformability, inferences about it may be drawn from data on cellular morphology obtained from a Coulter counter; internal red cell viscosity, a component in cell deformability, is related to mean corpuscular haemoglobin concentration (143,144). Data on cellular morphology for the TIA and control groups are shown in table 8.4; no differences are apparent between groups.

8.4 DISCUSSION

Viscosity changes associated with peripheral vascular disease are well documented. Increased blood viscosity in claudicants was noted as long ago as 1973 and was related at that time to an adverse clinical outcome (114,115). Elevated haematocrit, raised serum fibrinogen and increased red cell rigidity are all believed to contribute to abnormal viscosity in peripheral vascular disease (172,173). The difference between the claudicant and control groups presented here (Tables 8.2 & 8.3) are in keeping with previously published observations (114). Whole blood viscosity at both shear rates is significantly higher in claudicants than in controls. Correction of viscosity to a haematocrit of 45% reduces the differences, confirming that the higher haematocrit of the claudicants is responsible for some of the disparity. Plasma viscosity is also significantly higher in the claudicants and this contributes to the residual differences in whole blood viscosity after correction for haematocrit. Fibrinogen is a major determinant of plasma viscosity and the higher levels found in the claudicants are at least partly responsible for their observed elevation in plasma viscosity.

There is essentially no difference in the whole blood viscosity between the TIA group of patients and the controls. This is perhaps surprising if we consider that arterial disease is the underlying abnormality in the majority of these patients. While the extent or degree of their arterial disease may not be as marked as it is in claudicants, they might have been expected to show changes in blood viscosity which tended towards those exhibited by the claudicants. That the haematocrit of the TIA patients is identical to the control group is also surprising in view of the published observations of Harrison et al (174). In a retrospective study of 154 patients with TIA they discovered that haematocrit was slightly, but significantly, raised compared with controls assessed similarly. This difference persisted when allowance was made for the differing incidences of hypertension and smoking, both of which may affect haematocrit (136,175), in the two groups. There are several possible reasons for this discrepancy. Harrison's series of TIA patients contained 19 males (17.4% of males) with haematocrits >49%, while the present study had only two such patients (6.4% of males). In view of the widely recognised risk of raised haematocrit, it is possible that patients with abnormally elevated haematocrits were not referred for Doppler examination, at least until the PCV had been lowered to more physiological levels; this would introduce bias to the selection of patients in this series. On the other hand Harrison's study was carried out retrospectively with all the limitations which that imposes on data collection and interpretation. Other controlled studies of haematocrit in TIA are lacking.

While haematocrit and whole blood viscosity in TIA patients show no differences from controls, table 8.3 indicates that plasma viscosity and fibrinogen, important determinants of whole blood

viscosity, do show statistically significant differences. Since fibrinogen contributes disproportionately to measured plasma viscosity, it is likely that the rise in plasma viscosity observed in TIA patients is due partly or wholly to the abnormal fibrinogen levels. The fact that increased plasma viscosity is not reflected in increased whole blood viscosity means that other determinants of viscosity, red cell aggregation and deformability for example, must compensate for the raised plasma viscosity and fibrinogen levels.

The relative low and high shear viscosities shown also in table 8.3 indicate as much. These figures suggest that erythrocytes in the TIA patients deform more and aggregate less than cells from control patients. The increased deformability may be due to extrinsic (plasma) factors or intrinsic (cellular) factors. Mean corpuscular haemoglobin concentration is a major determinant of internal cellular viscosity (103) and, in the absence of accurate, direct measurements of red cell deformability, may be utilised as a measure of cellular flexibility. The figures presented in table 8.4 fail to show significant morphological differences between the red cells of TIA and control patients and it is possible to infer that increased deformability is probably due to plasma, rather than cellular, factors. Thus the increased plasma viscosity due to raised fibrinogen levels produces more deformation of the suspended red cells and the net effect on whole blood viscosity is nil.

The apparent reduction in red cell aggregation in TIA patients compared to controls indicated by the relative low shear viscosities (RCA) in table 8.3 is interesting. Fibrinogen contributes to blood viscosity in two ways: firstly via its contribution to plasma viscosity and secondly by virtue of its ability to form cross links between red cells and encourage aggregation. The elevated plasma

fibrinogen levels of the TIA patients might be expected to lead to enhanced rather than diminished aggregation. This paradoxical result may be an artefact of low shear viscosity measurement. Although the time taken to complete both viscosity measurements on a sample is only about six minutes, and the blood is mixed during the high shear programme, the delay between high and low shear measurements may be sufficient to allow sedimentation and separation of the sample and result in a falsely low viscosity reading in the presence of high fibrinogen concentrations.

It appears that all the differences between the control and TIA groups may be accounted for by raised fibrinogen levels in the latter group. This may be related to the greater numbers of smokers and hypertensives in this group, since these are both factors shown to be associated with elevated fibrinogen levels. The relationship between fibrinogen levels, arterial disease and these two shared risk factors is complex and is considered in chapter 9.

8.5 SUMMARY

High blood viscosity is known to be associated with stroke and other vascular disorders, and manipulation of viscosity may be beneficial in these patients. The results of the studies presented in chapter 7 suggested that TIA patients may also have abnormal viscosity and therefore an analysis of viscosity and its main determinants in these patients was undertaken. The results were compared to viscosity values from matched populations of patients with claudication and normal controls.

Whole blood viscosity is elevated in claudicants compared to the other two groups and this is due to a combination of raised haematocrit, increased plasma viscosity, and elevated plasma fibrinogen. No differences in whole blood viscosity have been demonstrated between TIA patients and controls, but plasma viscosity and fibrinogen are significantly higher in TIA patients. These differences are not reflected in differences in whole blood viscosity due to a compensatory increase in red cell deformation in TIA patients, which appears to be due to extrinsic (plasma) factors rather than cellular factors. The cause of the elevated fibrinogen levels in TIA patients is unclear, but it may be related to the increased prevalence of smoking and hypertension in this group.

Chapter 9

FIBRINOGEN: ITS RELATIONSHIP TO ATHEROMA AND CARDIOVASCULAR RISK FACTORS

9.1 INTRODUCTION

Fibrinogen is an acute phase reactant and is elevated in response to infection, malignant disease, surgical operations and "stress". It may also be elevated in response to more chronic, less obvious stimuli and it is in the context of these influences that the changes discussed in the last chapter must be considered. Epidemiological and clinical studies have related fibrinogen levels to age, smoking and blood pressure in healthy individuals.

The increasing levels which occur with age may be related to the increased prevalence of subclinical disease in older individuals or to the ageing process itself (137,142,146).

The elevations which occur in smokers are related to cigarette rather than pipe or cigar smoking (137,163) and may be partly reversible; individuals who desist from smoking have lower levels than current smokers but not as low as those who have never smoked (143). The reason for this association has not been established.

Systolic and diastolic blood pressure have been shown to be significantly correlated with plasma fibrinogen and plasma viscosity in a study in healthy middle aged men (176). Perhaps more relevant, in the context of cerebrovascular disease, is that many studies have

delineated a relationship between hypertension and blood rheology (103). This is due to increased haematocrit and plasma protein concentration. The correlation of hypertension with haematocrit is weak because although plasma volume is contracted, tending to increase haematocrit, renal disease, consequent on the raised blood pressure, may produce anaemia which opposes this change (177). A much greater correlation between whole blood viscosity and blood pressure is observed if measurements are corrected to a haematocrit of 45% (178) or if comparisons are made with a normotensive population with matched haematocrits (136). The increase in viscosity which is observed then is largely due to raised fibrinogen levels. There is no correlation between the viscosity of defibrinated blood and arterial pressure (136). Contracted plasma volume may explain many of the viscosity alterations in hypertension, but the increase in fibrinogen is disproportionally large and may represent a chronic phase reaction (179).

The increased incidence of hypertension in cerebrovascular disease in general and among TIA patients in particular is well documented (3,174) as is the association of smoking with arterial disease. The possibility that the changes in plasma fibrinogen noted in the studies reported in the chapters 7 and 8 have arisen as a result of these previously recognised associations must be considered. To explore this possibility the data obtained in these studies was subjected to sub-group analysis to look for evidence that the observed elevation in plasma fibrinogen in TIA patients was independent of associations with hypertension and smoking.

9.2 METHODS

Smoking habits were classified in three categories as previously described: non-smokers, current smokers and former smokers. This last group was defined as those who had stopped for at least three months. No attempt was made to subdivide smokers according to the quantity of tobacco consumed. The TIA and control groups from the study described in the previous chapter were divided into sub-groups on the basis of this smoking classification, and the mean fibrinogen levels calculated for each group. In addition, the TIA patients were further subdived, according to the findings on Doppler examination of their carotids, into Doppler-normal and Doppler-abnormal.

The effect of blood pressure was assessed in two ways. Firstly the control and TIA groups were subdivided on the basis of the presence or absence of hypertension. This was defined, as before, as the finding of a diastolic pressure greater than 90mmHg or the current prescription of anti-hypertensive medication. Mean fibrinogen levels for each of these sub-groups were calculated and the results compared. The second method of assessing the influence of blood pressure was to correlate it with whole blood viscosity and its determinants, in all patients not receiving anti-hypertensive drugs. Systolic and diastolic pressures were correlated individually with haematocrit, high and low shear viscosity corrected and uncorrected, plasma viscosity and plasma fibrinogen. The linear coefficient of correlation (r) was determined for each analysis (MINITAB) and its significance inferred by reference to standard tables.

9.3 RESULTS

Smoking

Mean plasma fibrinogen levels for each of the smoking sub-groups in the control and TIA groups are shown in table 9.1. It can readily be seen that in each of the smoking sub-groups the values for TIA patients are higher than those found in control patients. The effect of smoking is obvious within each of the two diagnostic groups; values among smokers and ex-smokers being higher than those in non-smokers, albeit only marginally in the control group.

Table 9.2 details the fibrinogen values found in each of the two Doppler sub-groups of the TIA patients, divided on the basis of their smoking habits. The highest levels are found in the three sub-groups of the Doppler-abnormal group, ie, in patients with detectable carotid disease. Again, in both diagnostic groups, values for smokers and ex-smokers are higher than for non-smokers. In each of these tables it appears that smoking and the presence of arterial disease have a modest additive effect on fibrinogen levels; the highest levels are found in patients who have a positive smoking history and greatest evidence of arterial disease, and the lowest levels in non-smokers who are asymptomatic.

Hypertension

A comparison of the control group and all TIA patients with regard to hypertension, shows (table 9.3) that the highest plasma fibrinogen levels occur among the TIA patients irrespective of the presence of hypertension. Among the control patients, however, the recognised relationship between fibrinogen levels and hypertension is apparent. It is worth noting that the fibrinogen levels in

	Control	TIA
Non-smokers	2.80 ± 0.28	3.17 ± 0.25
Current smokers	2.92 ± 0.17	3.88 ± 0.17
Ex smokers	3.00 ± 0.47	4.16 ± 0.44

Values are g/l ± SEM.

TABLE 9.1

PLASMA FIBRINOGEN LEVELS IN CONTROL SUBJECTS AND TIA PATIENTS CLASSIFIED BY SMOKING HABIT

	Doppler normal	Doppler abnormal
Non-smokers	3.05 ± 0.26	3.86 *
Current smokers	3.82 ± 0.29	3.95 ± 0.19
Ex-smokers	3.56 ± 0.58	4.88 ± 0.56

Values are g/l ± SEM.

* single measurement

TABLE 9.2

PLASMA FIBRINOGEN LEVELS IN TIA PATIENTS CLASSIFIED BY DOPPLER RESULT AND SMOKING HABIT

	Control	TIA
Normotensive	2.68 ± 0.13	3.93 ± 0.18
Hypertensive	3.62 ± 0.24	3.68 ± 0.22

Values are g/l ± SEM.

TABLE 9.3

PLASMA FIBRINOGEN LEVELS IN CONTROL SUBJECTS AND TIA PATIENTS CLASSIFIED BY BLOOD PRESSURE

	Doppler normal	Doppler abnormal
Normotensive	3.64 ± 0.22	4.55 ± 0.33
Hypertensive	3.50 ± 0.37	3.85 ± 0.25

Values are g/l ± SEM.

TABLE 9.4

PLASMA FIBRINOGEN LEVELS IN TIA PATIENTS CLASSIFIED BY DOPPLER RESULT AND BLOOD PRESSURE

asymptomatic hypertensives (controls) are similar to those found in Doppler-normal TIA patients. This perhaps suggests that sub-clinical arterial disease exists in both of these groups.

In chapter 6 it was observed that a slightly higher proportion of the Doppler-abnormal patients were hypertensive (diastolic blood pressure >90mmHg) or were receiving antihypertensive medication compared with the Doppler-normal group. The mean fibrinogen levels in each of these subgroups are shown in table 9.4. In both the Doppler-normal and abnormal groups the hypertensive patients have lower levels than the normotensive individuals, which is the reverse of what might have been expected. In both normotensive and hypertensive patients however, fibrinogen levels are higher in those who have carotid disease detected by Doppler scanning.

Table 9.5 shows the linear correlation cefficients and their level of significance for blood viscosity measured against systolic and diastolic blood pressure. No significant relationships have been demonstrated, except between plasma viscosity and systolic pressure.

9.4 DISCUSSION

The studies in this thesis were set up firstly to observe rheological differences between Doppler-abnormal and Doppler-normal TIA patients, and secondly to compare TIA patients with controls. In the first study, as the patients were consecutive, no attempt was made to match the groups in terms of age, smoking habits, or the incidence of hypertension. In the second study, control subjects were selected in such a way that age and smoking habits were roughly matched between the two groups. This matching however, was not perfect and with regard

	Systolic	p value	Diastolic	p value
Whole blood viscosity:				
-high shear native	-0.026	NS	0.130	NS
-high shear corrected	0.061	NS	0.079	NS
-low shear native	-0.051	NS	0.160	NS
-low shear corrected	-0.010	NS	0.124	NS
Haematocrit	-0.078	NS	0.072	NS
Plasma viscosity	0.215	<0.05	0.025	NS
Plasma fibrinogen	0.188	<0.1	0.025	NS

TABLE 9.5

CORRELATION COEFFICIENTS(r): BLOOD PRESSURE AND VISCOSITY

to hypertension was not performed. In an effort to allow for the differences in distribution of these important variables the analysis described in this chapter was carried out.

This kind of sub-group analysis is imperfect, firstly because the groups have not been selected prospectively and matching is incomplete, and secondly because the numbers in some sub-groups are small. Fortunately elaborate statistical manipulation has not been necessary. Subdivision of the various groups readily demonstrates that the biased distribution of smoking habits and incidence of hypertension does not account for the observed differences in fibrinogen levels. Indeed, among the TIA patients, those classified as being hypertensive had lower plasma fibrinogen levels than normotensive individuals.

The absence of any statistically significant correlation between systolic and diastolic blood pressure measurements and any of the whole blood viscosity measurements in this study is at variance with previously published studies, which have shown a relationship between blood pressure and blood viscosity (177,178). This is perhaps explained by the fact that the numbers studied in this thesis are smaller than in studies which have been specifically designed to look at this relationship. Furthermore relationships which exist in an asymptomatic population sample may not be entirely relevant in the presence of symptomatic arterial disease. Plasma viscosity did correlate with systolic pressure in this analysis however, and this may be partly due to plasma fibrinogen levels whose correlation with systolic pressure measurements just failed to reach a signifcant level. These findings are in keeping with previous studies which suggest that a positive correlation exists between arterial pressure and fibrinogen levels (136).

Although the relationship with hypertension was not quite as expected the relationship between smoking and plasma fibrinogen changes is more consistent with previously reported findings (137,180). Smokers and former smokers both have higher levels than patients who have not smoked, although the reversible nature of the influence of smoking has not been clearly demonstrated. The levels found in former smokers are statistically indistinguishable from those in current smokers. This disagrees with data previously published by Lowe and Kannel but it may be explained by the comparatively smaller numbers studied here (137,180).

Having established that increased fibrinogen levels are associated with TIA and detectable carotid stenosis independently of the risk factors of smoking and hypertension, the implications of this relationship remain to be explained. The link between arterial disease and raised plasma fibrinogen may be due to common aetiological factors and therefore coincidental, or each may be a consequence of the other. From a therapeutic standpoint the most interesting possibility is that raised fibrinogen produces arterial disease. If this were the case then reduction of fibrinogen levels would be expected to produce a decrease in the subsequent risk from arterial disease.

Abnormal plasma fibrinogen levels may theoretically contribute to clinically evident arterial disease in several ways. Firstly increased plasma and blood viscosity may be implicated in the mechanical initiation of atheroma. Secondly, fibrinogen is incorporated into atheromatous plaques and this process may be enhanced in the presence of raised plasma levels. Finally, raised fibrinogen levels may be a manifestation of a wider haemostatic disorder which predisposes to arterial thrombosis and embolism.

There is dispute about the precise rôle of flow mechanics in the production of atheroma. There is no doubt however, that it does play some part as atheroma is clearly a localised disease, which occurs in regions of high pressure and altered flow patterns, eg, the abdominal aorta and major bifurcations. It seems likely that the distribution of eddies, reverse flow, high and low shear stress regions, and other manifestations of turbulent flow, which occur at bifurcations, is affected not only by vascular geometry but also by the mechanical characteristics of blood. Factors which alter viscosity could thus have an effect on the mechanical forces which appear to be relevant in the initiation of atheroma (103).

Atheromatous plaques accumulate fibrinogen. Knowledge of this has been used in some cases to detect arterial disease by observing the incorporation of radio-labelled fibrinogen into carotid plaques (181,182). Whether this is purely a secondary event or is important in the development of arterial plaques is uncertain and the relevance of a raised fibrinogen level to this event is equally unclear.

The final method by which high fibrinogen levels may exert an influence on the subsequent development of arterial disease is via enhanced coagulation. Many of the clinically evident effects of atheroma are related to superimposed thrombus formation, either at the site of the arterial lesion or more distally following embolism. Fibrinogen enhances both red cell and platelet aggregation and an increased level will clearly contribute to thromboembolism (179).

Several epidemiological studies have related elevated fibrinogen levels to adverse events in cerebrovascular and coronary artery disease (135,180,183) and at least one study has correlated fibrinogen levels with extent of coronary atheroma (184). Kannel indicated that this effect was independent of any association with smoking habit and

suggested that the effect of smoking in arterial disease might be produced by its ability to raise the plasma fibrinogen (180). If fibrinogen is indeed central to the development of atheroma then clearly therapy aimed at lowering its level in individuals at risk could have substantial benefit.

9.5 SUMMARY

Sub-group analysis of TIA patients and control subjects was undertaken to determine whether the changes observed in plasma fibrinogen reported in chapters 7 and 8 could be explained by differences in smoking habits and incidence of hypertension among the groups. Initial analysis of the sub-groups clearly indicates that neither of these factors accounts for the observed changes. In conclusion it appears that elevated fibrinogen levels are associated with clinically evident arterial disease, the presence of hypertension and a positive smoking history; the most significant of these three influences appears to be the finding of clinically evident arterial disease. If elevated plasma fibrinogen is implicated in the production of arterial disease then pharmacological attempts to lower it in selected individuals may be rewarding.

Chapter 10

CONCLUSIONS

10.1 INTRODUCTION

The management of transient ischaemic attacks associated with carotid artery disease is a controversial topic. This chapter will attempt to draw together the findings of this thesis and discuss them in the context of these controversies. The first section will consider the investigation of patients with TIAs and the second part will look at the treatment of different groups of patients categorised by presence or absence of carotid artery disease. Section three will consider the possible rôle of rheological therapy in patients with cerebrovascular disease. The final section will discuss ideas for future research which have been generated by the findings of the studies reported in this thesis.

10.2 INVESTIGATIONS

The diagnosis of transient ischaemic attacks depends largely on accurate and complete history taking. Once this has been done, a full physical examination should be carried out, and in an effort to discover the likely cause of an attack, particular attention should be paid to examination of the cardiovascular system. Examination of the pulse and of the precordium should be carried out carefully in an effort to detect abnormalities which may indicate underlying valvular disease. Examination of the peripheral pulses on both sides may indicate evidence of widespread vascular disease. Measurement of the blood pressure in both arms is important not only to detect hypertension, but to search for any abnormality which might indicate disease of the proximal great vessels. Cardiovascular examination should also include careful examination of the optic fundi which may, in unusual circumstances, provide evidence of embolic disease.

Standard haematological and biochemical blood tests may yield important information in patients with TIAs. A full blood count may reveal polycythaemia or a high-normal haemoglobin which may be relevant. Abnormally large platelet counts may also be of diagnostic importance. Bearing in mind the well demonstrated association of coronary artery disease and transient ischaemic attacks, the estimation of fasting blood lipids is of value. A random blood sugar should also be measured.

Other investigations which should be carried out on all patients include a 12 lead ECG to search for other evidence of myocardial ischaemia, valvular disease, or arrhythmia. If this investigation, or any aspect of examination of the cardiovascular system, reveals evidence of cardiovascular disease, an echocardiogram and 24 hour

ambulatory ECG monitoring should also be performed (185,186). The increased incidence of unsuspected valvular disease in young patients suggests that an echocardiogram should be carried out in all patients under the age of 45 years regardless of the presence of signs, or positive investigations indicating myocardial disease. All patients should have cerebral computed tomograpy to exclude intracranial disease which may mimic TIA.

Non-invasive evaluation of the carotid circulation is indicated in all patients. A number of accurate methods are now in use and the best of these appear to be combined tests: occuloplethysmography and carotid phonoangiography; or Doppler imaging and spectrum analysis; or Duplex scanning. Each of these routines is sufficiently accurate to detect haemodynamically significant carotid stenosis in more that 90% of cases. It is probably not important which of these investigations is utilised, provided the investigators have sufficient experience in the application and interpretation of the tests. It seems likely that Duplex scanning, a combination of real time ultrasonography and spectrum analysis will provide the best screening method for carotid disease in the future. The ability of this test to discriminate between fibrous, calcified, and haemorrhagic plaques may allow patients to be categorised differently with regard to risk of recurrent stroke (45,98).

In patients who have positive carotid screening tests, and in whom surgery is contemplated, carotid angiography should be carried out. This is still most often done by a conventional intra-arterial injection but some centres now use digital subtraction angiography. This does not carry the risks associated with intra-arterial injection but its resolution is less than that of conventional angiography.

Intra-arterial angiography should be carried out by experienced radiologists if an acceptably low complication rate is to be achieved (70).

10.3 TREATMENT

In most series a majority of patients with carotid territory TIAs have disease of the internal carotid artery (34,50,82-85). If their carotid stenosis is haemodynamically significant and ipsilateral to their syptoms then these patients are candidates for carotid endarterectomy, assuming that the lesion is surgically accessible.

The association between carotid stenosis and subsequent stroke is complex. It is apparent that a symptomatic, high grade stenosis predisposes to a high risk of stroke, while an asymptomatic stenosis of similar degree or a low grade symptomatic lesion carries a much lesser risk. This may be explained by the fact that a large plaque which causes a high grade stenosis is more likely to be unstable and associated with thrombus formation. Conversely, a smaller plaque or a large asymptomatic plaque is likely to be more stable and less likely to be associated with thrombus formation and subsequent emboli. It is likely that, as our understanding of plaque morphology and related stroke risk increases, surgical treatment will be offered not simply for symptomatic high grade stenosis but for stenosis related to specific types of plaque. Usual practice in the UK at present however, is to confine surgical treatment to patients with symptomatic stenosis of at least 25% (152).

The relative merits of surgical and medical therapy are being studied at present in a large multinational randomised trial of carotid endarterectomy and aspirin therapy. While the results will not be known for some time yet it is possible to speculate on the likely conclusions which will be drawn from the trial. The difference between the benefits of surgery and medical therapy is likely to be small and the conclusion may be that if surgery can be carried out with a morbidity and mortality below a certain level then it should be preferred to medical therapy. On the other hand surgery with a higher morbidity and mortality is likely to be less beneficial than medical therapy. This will mean that carotid surgery should probably only be carried out in recognised centres where many operations are done and appropriate expertise is available. Although the benefits of aspirin therapy in TIA and stroke have not been conclusively demonstrated the available evidence suggests that a reduction in stroke rate of up to 16% is likely (187-189).

With regard to patients who have minimal carotid disease there is little argument in the UK that at present they should be managed conservatively with medical therapy. These patients should probably be kept under review using non-invasive carotid testing to monitor progress of their carotid plaque. Any increase in plaque size or recurrence of symptoms might be an indication to reconsider surgical intervention. At present there is insufficient information on the natural history of carotid plaque and its modification by drug therapy to speculate on the ideal management of these patients.

In patients in whom a contralateral stenosis is found, ie, an asymptomatic stenosis, surgery is not indicated. Although a great number of operations for asymptomatic carotid stenosis are carried out annually in the United States (151), this approach has not found

favour with vascular surgeons in this country (152). Asymptomatic carotid bruits are associated with increased risk of stroke in men over 45 years of age, but the side of the stroke correlates poorly with the side of the bruit. In addition there is a correlation with increased risk of myocardial infarction in the same group, indicating that such bruits are more important as indicators of generalised arterial disease than as harbingers of ipsilateral stroke (190). A recent study however, has suggested that an asymptomatic stenosis greater than 50% is associated with an increased incidence of ischaemic cerebral events (191). Until the natural history of asymptomatic stenosis is clearly defined and the stroke risk evaluated, it will not be possible to evaluate the likely benefits of surgery in these circumstances. However as already observed, it is possible that with improved understanding and recognition of plaque morphology, sub-groups of patients more likely to benefit from surgery will be identified.

The final group of patients to be considered in are those in whom no cause for the TIA can be found. The follow-up studies presented in this thesis (Chapter 6) indicate that such patients do not necessarily have a benign prognosis. The finding of an elevated fibrinogen level in these patients suggests that undetected arterial disease may be the basis of their symptoms. These patients should probably be treated along lines similar to those in whom non-haemodynamically significant arterial disease is detected. Management should be initially by medical therapy, with routine review using non-invasive carotid imaging. The subsequent appearance of haemodynamically significant lesions should be regarded as a reason to reconsider surgical

intervention and recurrent transient symptoms should perhaps be an indication for more aggressive investigation in the form of arteriography.

10.4 RHEOLOGICAL THERAPY

Rheological therapy may be directed at one or more of the principal factors which influence blood viscosity, ie, haematocrit, plasma viscosity, red cell deformability and red cell aggregation. The possible therapies which could be directed at each of these determinants and their likely benefits in patients with cerebrovascular disease will now be considered in turn.

Haematocrit

As was observed earlier in this thesis several studies have indicated that elevated haematocrit may be a risk factor for stroke (125) and TIA (174). However, more recent studies have indicated that the relationship between haematocrit and cerebrovascular events may be more complex than was initially appreciated. In a secondary study on the patients included in the co-operative study of intracranial-extracranial bypass, Wade was unable to demonstrate any increased risk of cerebrovascular events in patients with high-normal haematocrits compared to those with normal levels (192). He was equally unable to demonstrate, among those who had strokes, that those with high haematocrits were more severely disabled. This is clearly at odds with Harrison's previous study which demonstrated that in

patients with carotid occlusion, the volume of cerebral infarct measured by CT scan was related to the height of the haematocrit (129).

This apparent difference between these studies may be due to the fact that all the patients in Wade's study were treated with aspirin. This raises the possibility that the effect of haematocrit is not related to blood viscosity but rather to its effect on platelet function. As discussed in chapter 3 the mechanics of bulk blood flow dictate that erthrocytes occupy axial positions and platelets are displaced to the periphery of the flow, the high shear areas close to the vessel wall. As the haematocrit increases, this tendency of platelets to be displaced laterally also increases and they are consequently more prone to damage in these high shear areas. High shear damage to platelets can induce platelet aggregation and initiate thrombus formation. It is possible that the effect of haematocrit is thrombogenic rather than viscosity related. This would account for the abolition of the effect of haematocrit in Wade's study in relation to the prescription of aspirin.

The introductory part of this thesis drew attention to the initial encouraging results from a number of studies of haemodilution in acute stroke (138-140). Very recently the results of two extensive, multi-centre, randomised, studies of haemodilution in Sweden and Italy have been reported.

In the Scandinavian study, co-ordinated by Asplund, almost 400 patients were randomised to treatment and control groups (193,194). The protocol for the treatment group is shown in table 10.1. When the control and treatment groups were compared at three months there was no difference in mortality and this reflected the results found in the first pilot study (140). However, after the same interval, the

Day 1	Hct 38-42%	Venesect 250ml + infuse 500ml dextran
	Hct > 42%	Venesect 500ml + infuse 500ml dextran
Day 2	Hct 38-42%	Venesect 250ml + infuse 500ml dextran
	Hct > 42%	Venesect 500ml + infuse 500ml dextran
Days 3-5		Dextran 500ml

TABLE 10.1

ISOVOLAEMIC HAEMODILUTION PROTOCOL: SWEDISH MULTICENTRE STUDY

neurological scoring among the survivors was also no different between the groups, and this was at variance with the previously reported results (140). It appears that this regimen of haemodilution bestows no benefits. In fact, when the two groups were compared with regard to the total number of cardiovascular events, including myocardial infarction, congestive heart failure and pulmonary thromboembolism, there was an excess of events in the treatment group.

The Italian multi-centre study was an even larger trial of haemodilution in acute stroke co-ordinated by Candelisi (195, Royal Society of Medicine, Forum on Haemorheology, London, September, 1987, personal communication). In this study there were over 600 patients in each group and the protocol for the treatment group is shown in table 10.2. When the two groups were compared at six months the mortality rates were similar.

These results are obviously disappointing following the encouraging results obtained from the initial studies. Possible reasons for failure of haemodilution to make a difference in these patients are firstly that the intervention is too late and delay between onset of stroke and the time of admission to hospital effectively prevents any therapy from being applied soon enough. The second possibility is that the reduction in viscosity obtained from these two protocols is too small. While venesection reduces haematocrit and therefore viscosity, infusion of dextran increases plasma viscosity and the net change in viscosity may not be large. We cannot be certain of this since viscosity was not measured in these studies either before or after haemodilution. Another possibility is that the reduction of haematocrit reduces oxygen delivery to such an extent that this offsets any benefit obtained from reduction in viscosity.

On admission	Hct ≥ 35%	Venesect 350ml + infuse 350ml dextran
6-12 hours post admission	Hct ≥ 35%	Venesect 350ml + infuse 350ml dextran
12-24 hours post admission	Hct ≥ 35%	Venesect 350ml + infuse 350ml dextran

TABLE 10.2

ISOVOLAEMIC HAEMODILUTION PROTOCOL: ITALIAN MULTICENTRE STUDY

In view of the disappointing results obtained from these trials it has been suggested that further studies should examine the possibility that only certain types of stroke will benefit from haemodilution and selection of cases for haemodilution may be necessary (196). In addition hypervolaemic rather than isovolaemic haemodilution may be necessary (196). However, it seems that more work requires to be done in laboratory models or small clinical studies before further large scale clinical trials are undertaken.

While it appears now that haemodilution following acute stroke does not work, at least in unselected series, the possibility that haemodilution may offer some protection in patients at risk of stroke must be considered. However, while Harrison demonstrated that patients with TIAs had an increased haematocrit compared to controls (174) the figures presented in this thesis do not agree. In addition Wade's study of haematocrit in patients with symptomatic cerebrovascular disease indicates that patients with higher haematocrits do not appear to fare worse than others if aspirin is prescribed (192). It seems likely, therefore, that haematocrit reduction in patients with symptomtic cerebrovascular disease is not indicated, except in cases of primary proliferative polycythaemia which is known to carry a 20 fold increased risk of stroke (124).

Fibrinogen

Although increased haematocrit has not been demonstrated in TIA patients it has been shown in this thesis that these patients do have elevated fibrinogen levels and furthermore that the magnitude of the elevation appears to be related to the degree of detectable arterial disease. These observations fit well with work done by other authors

which indicates that fibrinogen is a risk factor for stroke and coronary artery disease (135,180,183). Fibrinogen is related to smoking habit but recent data from the Framingham study have indicated that the risk associated with fibrinogen is present even when smoking habits are taken into account (180). It is possible that the deleterious effects of cigarette smoking are mediated in part by its effect on plasma fibrinogen levels.

The relationship between raised plasma fibrinogen and arterial disease is complex and not fully understood. An association has been convincingly demonstrated in epidemiological studies and although this does not imply any causative relationship, it is possible to speculate that such a relationship exists. The development of arterial disease may be encouraged by elevated fibrinogen in a number of ways. It may enhance endothelial damage by increasing shear stresses at susceptible points in the circulation, it may be incorporated in increased quantities into atheromatous plaques or it can lead to increased red cell and platelet aggregation, and initiate thrombus formation.

If we are to consider the likely benefits of therapy to reduce fibrinogen levels however, we must bear in mind the possibility that fibrinogen is simply a marker for arterial disease and is not itself directly implicated in stroke risk. In recognition of this, Stuart coined the expression "haematological stress syndrome" to describe haemostatic abnormalities which accompany arterial disease and which may arise as a result of it (197). Another difficulty when considering therapy to lower fibrinogen levels is the limited number of drugs available to do this, and the hazards associated with those in current use (198). In a model of acute global cerebral ischaemia in rats, Grotta examined the effects of acutely lowering fibrinogen levels by exchange transfusion. Although he was able to lower blood viscosity

significantly, no difference in outcome between treated and control animals was observed (199). Ancrod is a defibrinogenating agent which has been used to lower whole blood viscosity in the treatment of acute stroke. A small randomised study demonstrated minimal benefit in the ancrod treated group over the control group (200). Ancrod therapy is limited by the need for parenteral administration and the development of resistance due to antibody formation which occurs in some patients. Clofibrate lowers fibrinogen as well as lipids but in a large trial of this therapy in ischaemic heart disease there was an unexplained excess of non-cardiac deaths in the treatment group (201). Finally, pentoxifylline may reduce fibrinogen levels but it may also act via other rheological mechanisms and its mode of action has not been fully determined. In practice there is as yet insufficient evidence to suggest that lowering fibrinogen levels using any of these methods in patients at risk of stroke is desirable.

Red Cell Deformability and Aggregation

Several studies have suggested that red cell rigidity may be increased in stroke although Pollock demonstrated that red cell rigidity is not an independent risk factor (121). Recent work however, has indicated that what was thought to be increased red cell rigidity is actually an increase in leucocyte rigidity, and the relevance of this is uncertain (202). The figures obtained from the studies reported in this thesis indicate that in TIA patients red cell deformation is, if anything, increased and therefore therapy to improve this even further would appear unnecessary. One small randomised study reported that pentoxifylline, a drug which reduces red cell rigidity, was superior to aspirin and dipyridamole in

preventing recurrent symptoms in patients with TIA (203). However pentoxifylline, in addition to its action on red cell deformability, reduces fibrinogen levels, reduces blood viscosity and inhibits platelet aggregation; any beneficial effects could therefore be due to a number of factors (204).

Red cell aggregation is influenced largely by haematocrit and by plasma fibrinogen levels. No evidence exists to suggest that abnormal aggregation may independently contribute to stroke risk nor that it may be manipulated other than via alterations in haematocrit or fibrinogen levels.

Rheological Therapy - Summary

In conclusion it appears that apart from increased fibrinogen levels leading to an increased plasma viscosity, TIA patients have no rheological abnormalities which might be amenable to treatment. Until the relationship between elevated fibrinogen and cerebrovascular disease is better defined there is no indication to lower plasma fibrinogen other than by indirect means such as stopping smoking and controlling hypertension. It appears that rheological therapy has been largely unsuccessful so far in reducing stroke risk and limiting mortality and disability following the onset of acute stroke.

10.5 FURTHER STUDIES

The follow-up study reported in chapter 6 indicated that patients with a normal Doppler examination following a classical carotid territory TIA were at increased risk of developing a stroke

subsequently. It seems that close study of this group in a prospective fashion may yield clues to the likely aetiology of their attacks, particularly if repeated non-invasive imaging of the carotids is undertaken. A small study in these patients is under way at present, comparing the findings of Doppler imaging and spectral analysis with duplex scanning; early results indicate that both routines produce identical results. Longitudinal study of these patients, using one or both of these imaging techniques, may detect the development of significant atheroma which may account for their symptoms. Until the cause of their symptoms is adequately explained it would seem prudent to prescribe anti-platelet medication for all of these individuals. These studies could usefully be integrated with similar cohort studies of patients with non-significant carotid stenosis and those with asymptomatic stenosis. Only long term follow-up of groups of patients in this fashion will allow an understanding of the natural history of TIA and stroke and their association with extracranial arterial disease. It is lack of such epidemiological knowledge which prevents agreement on the most appropriate management of, for example, asymptomatic carotid stenosis.

The future of rheological studies in cerebrovascular disease seems less certain now than it did some months ago. A series of negative studies, the multicentre trials of haemodilution being the most striking examples, has produced doubt about the rôle of rheological factors in the pathophysiology of cerebrovascular disease. Nevertheless many questions remain to be answered. The principal rheological finding of this thesis was the significant elevation of fibrinogen levels in TIA patients and the association of this with detectable carotid disease. Longitudinal study of TIA patients with monitoring of their haemorheological parameters, particularly

fibrinogen, will indicate if any particular abnormality predisposes to stroke. A randomised trial of therapeutic defibrinogenation in patients with elevated fibrinogen levels would help to indicate if fibrinogen is simply a marker for arterial disease or is implicated in its pathogenesis. Unfortunately no satisfactory drugs are yet available for this purpose and such studies must be postponed.

Ten years ago an editorial in the Lancet, addressing the advances made in haemorheological studies, stated that "we still await proof that changes in viscosity precede clinical manifestations (of disease) and are the prime factor" (205). Despite the many discoveries of the past decade we may still say the same today.

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